Ecological zonation of benthic foraminifera in the lower Guadiana Estuary (southeastern Portugal)

Camacho Sarita[a], Moura Delminda[a], Connor Simon[ab], Scott David[c], Boski Tomasz[a]
[a]CIMA, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[b]School of Geography and Environmental Science, Faculty of Arts, Monash University, Clayton 3800, Australia
[c]Centre for Environmental and Marine Geology, Department of Earth Sciences, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1
Corresponding author: scamacho@ualg.pt; phone number: 00351 919 741 802
Other authors: dmoura@ualg.pt; Simon.Connor@monash.edu; dbscott@dal.ca; tboski@ualg.pt.

Abstract

The present study analyses the spatial and seasonal distribution of live benthic foraminiferal communities in the estuary of Guadiana, the fourth largest river on the Iberian Peninsula, and establishes, through statistical analysis, their relationships with a series of environmental parameters. Forty-four superficial sediment samples were collected along distance-to-sea and elevation gradients in winter and summer 2010. Fifty-three foraminifera species were identified along the intertidal margins of the estuary. Foraminiferal distribution reflected seasonal variation of environmental factors, whose relative importance varied according to species tolerances. Elevation in relation to mean sea level appeared to be the most important parameter controlling foraminiferal distribution, probably because it combines the effects of a series of other variables (i.e. organic matter, sediment texture, pH and temperature). In the highest marsh areas, where environmental conditions approach survival thresholds, only some agglutinated species are able to survive. In the lower intertidal zone, where subaerial exposure is diminished and environmental conditions are generally less variable, more diverse faunas, mainly composed of calcareous species, prevail. During winter, when fluvial discharge peaks, agglutinated species represent more than 80% of the total individuals. In summer, when marine conditions prevail, calcareous species become more

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competitive, increase their densities and expand into higher marsh zones and estuarine upper reaches.

In the estuary, three different foraminiferal assemblages are distinguished: i) *Miliammina fusca* assemblage, which dominates in unvegetated areas of the lower marsh and tidal flats of the mid-upper estuary; ii) *Jadammina macrescens* assemblage, which dominates in the highest marsh areas in the lower estuary; and iii) *Ammonia aberdoveyensis* assemblage, which dominates the areas of low marsh and tidal flats of the lower estuary.

Keywords: Guadiana Estuary, foraminifera, seasonal distribution, elevation, bio-indicators.

1. Introduction

Foraminifera are single celled eukaryote organisms that occupy a great diversity of habitats, from the deepest oceanic environments to the upper limits of the tidal zones in coastal wetlands. Most foraminifera possess a hard test which, after death, remains in the sediment where it may eventually fossilize. This particular character brings some advantages compared to many other environmental proxies because foraminifera leave a permanent record in sedimentary sequences, enabling the reconstruction of the environmental history of a site in the absence of the original physico-chemical baseline data (Scott et al., 2001). In estuaries, foraminifera may serve as bioindicators of great interest as they have short life cycles and react quickly to changes (Debenay et al., 2000). Being small and abundant, foraminifera are found in great quantities in small sediment volumes, enabling statistically reliable and economically attractive studies (Scott et al., 2001).

Most ecological studies of foraminifera have been carried out with the aim of providing a contemporary database with which fossil foraminifera can be compared and interpreted (e.g. Wang et al., 1985; Thomas and Varekamp, 1991; Cearreta, 1998; Duleba et al., 1999; Edwards and Horton, 2000; Li et al., 2000). Saltmarsh foraminifera, in particular, are useful tools for Holocene sea-level reconstructions (Scott and Medioli, 1978; 1980a; Horton and Edwards, 2006). Statistical studies, based on the distribution of benthic foraminifera in marine and estuarine environments, have also shown that these organisms can be successfully used to identify various ecological provinces, to detect environmental stress conditions and to monitor pollution (Albani et al., 2001). Nevertheless, before using foraminiferal assemblages as stress and pollution indicators in transitional environments, a precise understanding of their response to environmental variables is necessary in order to distinguish between anthropogenic stress and natural environment changes (Debenay et al., 2000). This requirement is particularly critical in estuaries and coastal lagoons that are subject to a complex interaction of numerous physico-chemical natural parameters, each presenting spatial and temporal variability, and because these environments are often exposed to various human impacts such as chemicals, including industrial pollutants and agricultural pesticides (Debenay, 1995; 2000; Debenay et al., 2000).

Explaining foraminiferal distribution patterns thus requires consideration of a broad range of environmental factors (Murray, 2001). Among the many parameters studied in marginal environments, elevation, which controls the time of subaerial exposure between tidal cycles, is widely regarded as the principal factor controlling foraminiferal distribution (Scott and Medioli, 1978; Thomas and Varekamp, 1991; Nydick et al., 1995; Horton et al., 1999; Gehrels, 2000; González et al., 2000; Horton and Murray, 2007).

Other parameters, such as presence or absence of vegetation, desiccation, porewater salinity and pH have also been identified as important ecological controls on marsh foraminifera (Murray, 2006). In the Great Marshes of Massachusetts, De Rijk (1995) and De Rijk and Troelstra (1997) found that foraminifera distribution was controlled mainly by spatial and temporal changes in a series of environmental variables, particularly salinity, leading them to conclude that there is no single model relating foraminifera to elevation, that can be applied worldwide. Likewise, Goldstein and Watkins (1998), in a study of the saltmarsh of St. Catherine’s Island, Georgia, found significant differences in the foraminiferal distribution patterns compared to those described by Scott and Medioli (1978; 1980a; 1986), which they explained by differences in geographical setting, including differences in saltmarsh physiography. In the mangrove swamps of French Guiana, elevation was found to have only minor influence on the distribution of foraminiferal assemblages (Debenay et al., 2004).

Species will be able to survive and potentially prosper as long as conditions remain within their tolerance limits. Once conditions move beyond the tolerance limits for any limiting factor, the species is likely to disappear (Murray, 2003). Summarizing, in estuaries, a great diversity of stress factors disturbs the living communities and causes significant temporal and spatial variability in the assemblages of benthic microfauna. As a result, the composition of benthic foraminiferal assemblages reflects the complex interaction between biotic and abiotic parameters and their multiple changes in space and time (Debenay et al., 2000).

In Portugal, baseline studies in the more pristine coastal ecosystems are rare and the use of foraminifera as indicators of environmental status has hitherto been relatively uncommon. In order to reverse this trend, the present work aims to identify the spatial-seasonal variation in the distribution of living benthic foraminiferal assemblages in the Guadiana Estuary and to investigate their relationships with environmental parameters; this is expected to improve the possible use of benthic foraminifera as bioindicators, provide baseline data for future inferences of environmental quality in the Guadiana, and to provide a modern analogue dataset for reliable paleoenvironmental interpretations on local and regional scales.

2. Study area

The Guadiana River rises in the Ruidera Lakes in Spain, at 1700 m altitude, and runs 810 km south until reaching the Gulf of Cadiz and the Atlantic Ocean, between the Portuguese town of Vila Real de Santo António and the Spanish town of Ayamonte (Fig. 1). Located between 37º and 40º N and between 2º and 8º W, its catchment area covers approximately 66 900 km^2 (Brandão and Rodrigues, 2000). The Guadiana Estuary makes part of one of the most important mesotidal fluvio-marine systems of the south-western Iberian Peninsula (Morales, 1997; Morales et al., 2006). The mean tidal range at the river mouth is approximately 2 m, reaching 3.4 m during spring tides (Instituto Hidrográfico, 2011) and the tidal influence extends approximately 44 km upstream (Oliveira et al., 2006). The estuary is funnel-shaped and filled with post-glacial sediments (Boski et al., 2006). Currently, it is in advanced state of sediment infilling, with the formation of a flood delta at its mouth caused by the interaction of coastal sedimentation processes and a relatively stable sea level (Morales et al., 2006). Its basin has Mediterranean climate characteristics, with hot, dry summers, and rainy cooler winters.

Insert Fig. 1

During the winter sampling in February-March 2010, high precipitation was observed, leading to high fluvial discharge, especially in March, when maximum discharge values of approximately 2100 $m^3 s^{-1}$ were registered. The summer was typically dry, with no rain and low, regular, discharges (mean $Q = 52 m^3 s^{-1}$) (SNIRH, 2012).

The Guadiana Estuary represents a rich area in terms of ecological interest, noteworthy for its endemism and halophytic saltmarsh communities. The Lower Guadiana River is listed as a Wetland of International Importance (Ramsar, 2013), is included in the Natura 2000 Network and its extensive marshlands were declared a Nature Reserve in 1975 (Instituto da Conservação da Natureza e Biodiversidade, 2007). Nevertheless, a number of environmental impacts have been reported in recent years (Morais, 2008; Guimarães et al., 2012), resulting mainly from untreated waste water discharge, agriculture and damming. The morphology of the estuary itself has been changed dramatically due to the building of hard engineering structures (dams and jetties) strongly conditioning the natural exchange between continent and sea.

3. Material and Methods

3.1. Sampling strategy

In 2010, forty-nine surface sediment samples were collected for benthic foraminifera analysis during two field campaigns in the two most contrasting seasons, winter (February and March) and summer (August). The sampling extended over 27 km, from Laranjeiras village to the mouth of the river Guadiana (Lagoa site – LG) (geographic coordinates for all samples are presented in Table A.1, Appendix A). In total, eleven sites were sampled, the majority located on the Portuguese side of the river (Fig. 1). In the middle and upper estuary, only one sample per site was collected due to the absence of saltmarsh zonation. In the lower estuary, where the environmental zonation is well-defined due to the stronger effect of the tidal range, several samples were collected along elevational profiles at each site, usually perpendicular to the main river channel. Transects were placed according to the vertical zonation of halophytic vegetation, with the aim of sampling the distinct saltmarsh zones and the unvegetated tidal mud-flats (Fig. 2). At the eleven different sites, five individual samples and six transects were collected, resulting in a total of 49 samples (24 in winter and 25 in summer). Site PI1, approximately 8 km upstream from the estuary's mouth, was only sampled in summer due to technical problems. Detailed topographic profiles were produced using a differential Global Positioning System (d-GPS), a Trimble 5800 mobile unit, and a Nikon DTM 310 Total Station. Elevation values were measured in relation to mean sea level (MSL), which is the adopted mean value for water level derived from a series of tide gauge observations of variable duration (Instituto Hidrográfico, 2011) (Fig. 2). The elevation (intertidal) gradient was divided in: 1) upper marsh zone (samples collected at 1–2 meters above MSL, mainly high marsh vegetation), 2) lower marsh zone (0–1 meters above MSL, mainly mid-low marsh vegetation), and 3) mud zone (-1–0 meters in relation to MSL, mainly unvegetated tidal mud-flats). The distance-to-sea gradient was divided into lower, middle and upper estuary (Fig. 1) according to Boski et al. (2006).

At each sampling point, two pseudoreplicates were collected (replicates at the same site, thus not statistically independent) (Hurlbert, 1984) with the aim of neutralizing the effects of patchiness (Fatela and Taborda, 2002; Debenay et al., 2006; Armynot du Châtelet et al., 2009), or non-uniform distribution of benthic communities (Underwood and Chapman, 2005; Morvan et al., 2006; Murray, 2006). In nature, most of the populations exhibit varying degrees of patchiness in response to biotic and/or abiotic factors, promoted by both natural and anthropogenic disturbances (Odum, 1997). In shallow, marginal environments, foraminiferal communities form a heterogeneous continuum trough time and space due to asynchronous reproduction pulses (Buzas et al., 2002). In the present case, each pseudoreplicate was collected using small PVC cores of 15-cm length and 5-cm diameter. Each core was pressed into the sediment to a depth of 10–15 cm, but only the first centimeter of sediment (19.6 cc) was kept for foraminiferal analysis. Sample collection and measurements of physico-chemical parameters were always performed during ebb tide. At each sampling point, ca. 300 g was collected in the uppermost part of the sediment (up to 3-cm depth) for bulk organic matter and sedimentological analyses. Additionally, salinity, temperature and dissolved oxygen in the sediment interstitial water were measured *in situ*, using an YSI 556 MPS handheld multiparameter probe. The pH was measured at the sediment surface with a waterproof portable EuTech (pHSpear) instrument. Results of physico-chemical parameters are compiled in Table B.1, Appendix B. Detailed results and methodologies are described in Camacho et al. (2014).

Insert Fig. 2

3.2. Laboratory procedures

Each pseudoreplicate for microfaunal analysis was sub-sampled (10 cc) and kept in a mixture of rose Bengal and ethanol (2g/l) for two days. After this period, the samples were washed over a column of two sieves, 63 µm to retain the fraction for analysis and 500 µm to remove the large sediment and organic debris. To obtain the final fraction (20 cc), the retained 63-500 µm fractions of two 10 cc aliquots were merged and counted. Whenever possible, counting was made in liquid, which favors the observation and identification of the stained parts, even of porcelaneous and agglutinated tests (Scott et al., 2001). Samples with few individuals or with calcareous tests only, were dried and treated with carbon tetrachloride to float off the tests. Samples with high organic content and abundant foraminifera, both calcareous and agglutinated, were subdivided using a wet splitter (Scott and Hermelin, 1993).

Several taxonomical reference studies were used for species identification, especially Loeblich and Tappan (1988), Colom (1974), Jones (1994) and the Ellis and Messina (1942–2012) online catalogue. A main reference collection, established by previous studies of Guadiana river paleoenvironments (Boski et al., 2002; 2008) was also used. Most of the foraminifera were classified accordingly to the generic classification proposed by Loeblich and Tappan (1988). For the higher levels of taxonomy, other than genus and species, the World Register of Marine Species classification was adopted (WoRMS, 2012). A few rare species were not determined at the species level, particularly among porcelaneous forms, for which ten species were recognized but left in *incertae sedis* with code names (e.g. Miliolid sp1, sp2, sp3 etc). In collaboration with the project foramBARCODING (Pawlowski and Holzmann, 2012), four of the main species were identified by genetic analysis, namely *Ammonia aberdoveyensis*, *Elphidium oceanenis*, *Elphidium williamsoni* and *Discorinopsis aguayoi* (Camacho, 2012). In the case of *A. aberdoveyensis*, three morphological variants (*Ammonia* sp1, sp2 and sp3) were analyzed but only two produced successful genetic results. Both sp2 and sp3 align with *Ammonia* T2 (Hayward et al., 2004), thus corresponding to the species *Ammonia aberdoveyensis* Haynes (1973) in the foramBarcoding.unige.ch database (Pawlowski and Holzmann, 2013). Further field collections were performed, but genetic analysis of *Ammonia* sp1 proved fruitless. Some morphological dissimilarity was observed between the three variants (Camacho, 2012) but no significant correlation of each of them with specific environmental conditions was found. Thus, awaiting new analyses to determine whether *Ammonia* sp1 is a different type or belongs to *A. aberdoveyensis* species, with morphological differences resulting mainly from intergradational variability, all the three variants were grouped in the species *A. aberdoveyensis*.

The most important foraminifera species were photographed using a scanning electron microscope (SEM), model JEOL JSM–5410, based at Huelva University, Spain.

3.3. Data analysis

Both living and dead assemblages were counted, although only the living fraction was assessed (Tables C.1 and C.2, Appendix C) to interpret the ecological meaning of the assemblages, i.e., the associations between species and their dependence of environmental parameters. Data on the dead assemblage will be used in future for the reconstruction of the paleo-estuary (Camacho et al., in prep.). Whenever possible, 100–300 living individuals were counted. All statistical analyses were performed using the relative abundance of living foraminiferal species with relative frequencies above 5%, or lower than 5% when present in more than 50% of the samples (‘constant species’ in Dajoz, 1978). Considering both seasons, 15 of the 49 samples (30.6%) had counts below 100 individuals. To avoid loss of information, a statistical threshold of 48 live individuals was established. Samples with less than 48 individuals were discarded from the data matrix for statistical analysis (in grey in Table C.1 and C.2 in Appendix C). In total, 42 samples were statistically analyzed. According to Fatela and Taborda (2002), counts of 100 individuals guarantee a probability of up to 99% of including all the important species ($\geq$ 5%), providing a reliable representation of the assemblage. With counts of 48 individuals this probability is diminished to approximately 92% and the relative frequencies present a binomial standard error (σ) of ± 3%.

Various biocenotic indices were calculated using PRIMER-E, version 5.2.0 (2001) for Windows: faunal density (Ni, number of individuals per 20 cc of sediment); number of species (S); species dominance (where species with more than 20% are considered dominant); agglutinated/calcareous (hyaline + porcelaneous) ratio (A/C% = A*100/Ni);

and Shannon index of diversity. These parameters were cross-correlated to highlight their main relationships using Pearson's or Spearman's coefficients, depending on the normality of the variables, which were tested through Shapiro-Wilks W test in STATISTICA, version 7.0 (StatSoft, 2004).

Three sets of environmental variables were considered for multivariate analysis: a) spatial variables – distance-to-sea (Dsea), measured in geographical latitudinal degrees (as river course is perpendicular to the Equator), and elevation, measured in meters in relation to MSL (Elev); b) chemical variables - salinity (Sal), total organic carbon (TOC), total inorganic carbon (TIC), carbon/nitrogen ratio (C/N), sediment pH (pH_{sed}) and dissolved oxygen (DO); and c) physical variables - granulometric mean (Gmean), sand, mud, gravel and temperature (TºC). Autocorrelation between environmental variables and their influence on foraminiferal species distribution were inferred through forward selection in Redundancy Analysis (RDA), a constrained linear ordination method. Estimation of community composition heterogeneity was performed by detrended correspondence analysis (DCA). Where the gradient length was shorter than 4 SD (standard deviation), this was taken to indicate that most species responses can be approximated with a linear model (Leps and Smilauer, 2003). RDA is a constrained form of the linear ordination method of PCA and was chosen to explore species–environment relationships. RDA's were performed with a focus on inter-species correlations. The species scores were divided by a SD, where the length of each species arrow on the ordination diagram expresses how well the values of that species are approximated. This representation corresponds to a correlation biplot (Legendre and Legendre, 1998) and the abundance of individual species is transformed to a comparable scale (Leps and Smilauer, 2003). A Monte Carlo Permutation Test was used to test the significance of the environmental variables in species distribution when using forward selection (manual and automatic) and to test the global model significance without forward selection, with a maximum number of 499 permutations under a reduced model. All tests were performed in CANOCO for Windows, version 4.56 (Ter Braak and Smilauer, 1997–2009).

4. Results

Results on physico-chemical parameters are presented in Table B.1, Appendix B.

4.1. Faunal density and diversity

In both seasons there was a clear decreasing trend in Ni (number of individuals/20 cc of sediment) with decreasing elevation (Fig. 3 and Table 1). High densities (Table 2) (reaching an extreme value of ≈76 000 individuals/20cc sediment in sample LG1 in winter) were found in samples with few agglutinated species in the most elevated areas of the lower estuary. Generally, in the upper marsh environments the densities were higher in winter than in summer. In low-marsh environments, higher densities were found in summer compared to winter (Fig. 3A). Foraminifera density is generally lower in the upper reaches of the estuary (Fig. 4A), but the trend of increasing density with decreasing distance-to-sea is not significant (Table 1).

Insert Table 1 and Fig. 3

On the intertidal margins of the Guadiana Estuary, S (number of species/20 cc of sediment) varied from 1 to 21 species. Along the elevation gradient, S exhibited an opposing trend to Ni (Table 1 and Fig. 3A and B), with fewer species in the upper marsh and more species in the low marsh (Table 2). Along distance-to-sea gradient, S follows on the same trend as Ni, with the highest values in the lower estuary (Fig. 4A and B) although the trend is not significant (Table 1).

Insert Table 2 and Fig. 4

In both seasons there was a general trend of increasing Hs (Shannon diversity index) with decreasing elevation, although slightly less pronounced in summer than in winter (Table 1). Hs was higher during winter (Fig. 3C) but like for S, no significant decrease was observed in Hs with increasing distance-to-sea (Table 1 and Fig. 4C), even when analyzing the group of middle elevation samples (0-1 m), where all samples of the upper reaches are included.

4.2. Agglutinated/Calcareous ratio (A/C%)

For both seasons, A/C% was highest at the upper elevations, where some samples were exclusively constituted of agglutinated species (A/C% = 100) (Table 2 and Figs.

3D and 4D). Towards lower elevations, the agglutinated forms are progressively replaced by more diversified calcareous assemblages. However, the correlation between A/C% and elevation in summer is stronger (Table 1) suggesting a lower dispersion of the agglutinated species towards the lower stands during this season. No obvious A/C% variation along the distance-to-sea gradient was found (Fig. 4D and Table 1).

4.3. Species /test type abundance, dominance and constancy

During both seasons, the same number of living species was counted: 43 in winter and 43 in summer (Table C.1 and C.2, Appendix C), amounting to 52 different species in the Guadiana Estuary study area. In winter, 55% of the taxa were calcareous, of which 21.4% were porcelaneous, and 45% were agglutinated. In summer, 49% of the taxa were calcareous, of which 11.6% were porcelaneous, and were 51% agglutinated (Fig. 5). In terms of number of individuals counted in winter, 81% were agglutinated and only 19% were calcareous, with 6.7% porcelaneous. In summer, total agglutinated individuals decreased substantially to 58% and the calcareous taxa increased to 42%, although with a decrease in the porcelaneous forms, which represent only 2.7% of the calcareous.

Insert Fig. 5

Relative abundances and constancy statistics were calculated for all living taxa in all samples (see Table C.1 and C.2, Appendix C). Common (10-20% abundance) and dominant species (> 20% abundance) were photographed in SEM and are illustrated in Plate I.

Insert Plate I

Of the living foraminifera taxa found in the Guadiana Estuary, 11 were dominant. Of those, only *Jadammina macrescens, Trochammina inflata, Miliammina fusca, Ammonia aberdoveyensis* and *Haynesina germanica* were dominant in both seasons. *Bolivina ordinaria,* Miliolid sp.3 and the miliolids group were only dominant in winter whereas *Polysaccammina hyperhalina, Polysaccammina ipohalina* and *Elphidium oceanensis* were only dominant in summer. Four species were common: *Asterigerinata mamilla*, *D. aguayoi* and *Miliammina obliqua* in winter and *Siphotrochammina* sp. in summer.

4.4. Environmentally-driven patterns in species distribution

The relationship between environmental variables and their importance in driving the biological distribution for each season was analyzed using Redundancy Analysis (RDA). The RDA's were performed using automatic selection, which gives the marginal (the independent effect of each environmental variable) and the conditional (the effect that each variable brings in addition to all the variables already selected) effects of each variable (Table 3). Among the winter set of environmental variables, only Elev and Dsea were significant when a 0.05 probability threshold level is adopted (values in bold in Table 3). Table 3 shows that, for the summer data, only Elev, Dsea and Gmean were significant among the variables, when a 0.05 probability threshold is adopted.

Insert Table 3

4.5. Winter ecological model

The winter final RDA was performed with only the two most important environmental variables: Elev and Dsea (Fig. 6a). Alone, Elev and Dsea were responsible for 36% of the total explained variance, of which 80% was explained on the first axis. Both axes presented a good correlation between species distribution and environmental parameters, r = 0.82 for the first axis and 0.84 for the second axis. The significance of the first and all axes, using Monte-Carlo permutation tests, was high (p = 0.002). In Figure 6a, sample scores were based on species composition (linear combinations of species in the samples; *Samp* scores in the CANOCO solution file) and the scaling was focused on inter-species correlations. Elev was correlated positively with the first axis (r = 0.72) and was responsible for the major division of samples into two groups: I and II. Dsea was positively correlated with the second axis (r = 0.81) and was responsible for a secondary division of group II into samples of lower elevations located in the lower estuary (group IIa) and samples of the lower elevations located in the upper part of the estuary (group IIb). Figure 6a shows that, with the increase of both environmental variables (Elev and Dsea), there was a decrease in diversity, with the majority of species concentrated at the lower elevations in the lower estuary (group IIa). Also, with the increase of either of the two variables, there was a decrease in calcareous species, since the most elevated and upper estuary environments were dominated by agglutinated species.

Insert Fig. 6

The interpretation of species distribution and assemblage trends can be enhanced with a shade matrix (Fig. 6b), wherein samples and species are ordered according to their position in the RDA with the original abundances represented by symbols of increasing size and density. In winter, the two most ubiquitous species were *Jadammina macrescens* and *Ammonia aberdoveyensis*. Their presence in almost all samples indicates their high degree of resilience to daily changes in environmental factors, typical of estuarine intertidal areas. *Jadammina macrescens* has its optimum in the upper elevations of the lower estuary, where it almost exclusively dominated the assemblage. In terms of distribution, *Jadammina macrescens* closely resembles *Trochammina inflata.* The latter species was present in almost all the samples in which *J. macrescens* also occurs, although it reached its highest abundances in the middle elevations where the *J. macrescens* abundance decreased slightly and diversity increased. *Ammonia aberdoveyensis* has its optimum in the lower to middle elevations of the lower and middle estuary and was systematically associated with *Haynesina germanica*. In samples located far from marine influence, *A. aberdoveyensis* and *H. germanica* were associated with *Miliammina fusca* and *Elphidium oceanensis,* whereas in samples located near the river mouth they were associated with *Bolivina ordinaria, Asterigerinata mamilla* and *Polysaccammina hyperhalina*. It is also worth pointing out the stenotypic distribution of some species, which occurred only in specific environments, sometimes in significant numbers. This was the case of *Miliammina obliqua* and *Discorinopsis aguayoi,* which occasionally occurs as subsidiary species to *J. macrescens,* as well as *Bolivina variabilis,* which had a high incidence in pond/soaked environments. The porcelaneous taxa (miliolids, Miliolid sp1 and sp2), did not exhibit strong gradients in their distribution, presenting large ranges in elevation and distance-to-sea. This suggests that the two most important environmental variables that explain the species general distribution do not have the same importance for the distribution of porcelaneous taxa.

4.6. Summer ecological model

The summer final RDA was performed with only the most important selected environmental variables: Elev, Dsea and Gmean (granulometric mean) (Fig. 7a). These variables were responsible for 57.2% of the total explained variance, of which 73.6% was explained by the first axis. The first and second axes represent a good correlation between species distribution and environmental parameters, r = 0.87 and 0.81, respectively. The significance tests on the first and all axes were highly significant (p = 0.002). The same rules of data projection for the winter data were used (Fig. 7a). Elev correlated positively with the first axis (r = 0.82) and is responsible for the major division of samples: groups I is positively correlated with Elev, whilst group II is negatively correlated. Dsea was positively correlated with the second axis (r = 0.70) and was responsible for a secondary division of the samples: group IIa was negatively correlated with Dsea, while group IIb was positively correlated. Gmean is negatively correlated with both axes, and consequently, with both Elev and Dsea.

Figure 7a shows that there were few species tolerant to increasing elevation and that these were all agglutinated. The majority of the species were concentrated in the lower elevations of the lower estuary, where calcareous taxa were dominant (group IIa). In samples from the middle elevations of the upper reaches (group IIb), agglutinated taxa dominate, although they are coexisting with calcareous forms.

Insert Fig. 7

The shade matrix (Fig. 7b) shows that in summer, as in winter, the two most ubiquitous species were *Jadammina macrescens* and *Ammonia aberdoveyensis*. The major difference between the two seasons is that *A. aberdoveyensis* had a higher dominance in summer, especially in the samples of group IIa. In middle elevation samples of the upper reaches (group IIb), *A. aberdoveyensis* was still very prominent, being present in all samples, but subdominant to *Elphidium oceanensis* and *Miliammina fusca*, which have their optimum there. *Jadammina macrescens* had its optimum in the upper elevations of the lower estuary, where it dominated almost exclusively.

Trochammina inflata was again subsidiary to *J. macrescens*, being present in almost the same samples as the latter species, but having a weaker expression when compared to its winter abundance. Noteworthy is the occurrence of some isolated species, such as *Polysaccammina ipohalina* at SCM and *Siphotrochammina* sp. at Aouro. Both taxa were exclusive for these sites, occurring only in summer with significant relative abundance values: 20.8 and 12%, respectively.

The porcelaneous forms, here represented by Miliolid sp1, sp4 and sp5, were not constant species and were restricted to lower to middle elevation samples.

5. Discussion

5.1. Context

The study of living assemblages provides baseline knowledge on ecological trends and distribution patterns that are essential for interpreting paleoassemblages. This knowledge is fundamental to understand and identify the post-mortem changes that could affect preservation (especially dissolution (calcareous) and disintegration (agglutinated) of tests, and hydraulic transport of exotic taxa) of both dead and total assemblages (Horton and Murray, 2007). To fully represent the paleoassemblages, monthly, or preferably fortnightly, modern samples should be collected, ensuring a complete record of biologic and ecologic variations during foraminiferal life cycles under changing ecological and environmental conditions (Debenay et al., 2006; Murray, 2003). Unfortunately, such studies are very rare due to the time consuming tasks of foraminifera counting and identification, and consequently most studies are based on single sampling periods (Horton and Murray, 2007). In these cases, total assemblages (live plus dead) are preferable as they integrate seasonal compositional changes in the fauna due to biotic and taphonomic processes, while living assemblages represent only a snapshot in time, highlighting the behavior of foraminifera in response to rapidly changing local conditions (Duchemin et al., 2005). In the present work, as the main objective was to increase the ecological information available in order to improve the use of benthic foraminifera as bioindicators in transitional environments, only the living assemblages were analyzed. The two most extreme seasonal periods, winter and summer, were sampled to gauge the major environmental differences. This goal was achieved, recording a very wet winter and a typical, hot summer and substantial faunal differences between the two seasons. Three to four samples were collected along several transects in zones where major halophytic differences occur. This relatively low sampling frequency is compensated by the broader spatial scale of the study, encompassing several transects and single points along a length of approximately 34 km of estuary. This sampling strategy was adopted to register a maximum of sub-environments along both, distance-to-sea and elevation gradients.

5.2. Spatial and temporal ecological trends

In the present work, biocenotic indices are used as descriptors in the characterization of the main foraminifera ecological trends in space (elevation and distance-to-sea gradients) for two contrasting seasons.

Both Hs and S show values typical of estuarine environments (see Murray, 2003), with Hs varying from 0.043 to 2.34 and S varying from 1 to 21 species. Diversity in the Guadiana Estuary is higher than in the northern Portuguese systems (Minho, Coura and Caminha estuaries), where Hs never surpassed 1.85 and S never surpassed 13 species. In these estuaries, fluvial dominance promotes such extreme conditions (low pH and low salinity being the most evident), that a considerable quantity of sites is barren of foraminifera and the existing assemblages are mainly agglutinated, with few calcareous occurrences (Moreno et al., 2005; Fatela et al., 2009).

In general, diversity increases from the intertidal zone to the outer neritic zone (Sen Gupta and Kilbourne, 1974; Douglas, 1979 and references herein). This trend was also observed in the Guadiana Estuary in relation to the adjacent continental shelf, where Mendes et al. (2012) found Hs varying between 1.1 and 3.2 and S varying between 13 and 68 species.

Changes in species diversity and dominance are potentially a measure of stressful conditions, with diversity decreasing and dominance increasing as the environment becomes more stressed (Odum, 1997; Murray, 2003). In the Guadiana Estuary, both diversity indices (Hs and S) show a similar decreasing trend with increasing elevation but no significant decrease was found with increased distance-to-sea. In the upper marsh, only a few species, mostly agglutinated, are able to survive the extreme and variable environmental conditions, i.e. low pH and oxygen concentrations, seasonally varying salinity and sediment desiccation/frosting during summers/winters. Such extreme conditions promote high levels of physiological stress, diminishing inter-species competition and favoring the better adapted species.

The correlation between HS and S, which is always positive due to its interdependence, is stronger in winter (R = 0.89) when evenness is higher, and consequently the numbers of rare species and species dominance are lower. Trends in both Hs and S with elevation and season suggest generally harsher environmental conditions in summer and in the areas subject to longer periods of subaerial exposure between tidal cycles.

Greiner (1974) presented evidence that $CaCO_3$ availability controls the distribution of shell types, suggesting a gradient in estuaries in which environments with low carbonate availability would be dominated by agglutinated foraminifera, areas with intermediate availability by hyaline foraminifera and settings with high availability by porcelaneous foraminifera (miliolids). $CaCO_3$ precipitation and dissolution is mainly governed by the environmental pH. In densely vegetated high marshes, sedimentary organic content is usually high and, as a consequence, pH is low and the $CaCO_3$ precipitation is hindered. Accordingly, the agglutinated/calcareous ratio (A/C%) may be tentatively considered as an indirect measure of $CaCO_3$ availability (fresh/marine water and continent/water gradients). In the present work, the A/C% ratio gradually increases from the lower zones to the high marsh in both seasons, although this trend is stronger in summer suggesting higher dispersion of the agglutinated forms towards the lower elevations during winter. Lower temperatures and higher freshwater input from rainfall during winter could decrease the capacity for $CaCO_3$ precipitation, which could be potentially favoring the dispersal of typically upper elevation agglutinated species to the lower levels of the intertidal margin. In fact, an overall dominance of agglutinated forms was observed during winter, where 81% of the individuals were agglutinated and only 19% calcareous. In summer there was an increase in the percentage of calcareous individuals (42%) and the relationship between elevation and A/C% was much better defined (R = 0.9), with very high values of A/C% in the most elevated and upstream samples and very low A/C% values in the lower zones of the lower estuary. These seasonal changes suggest more steady environmental conditions in the elevation end-members which may be favoring the calcareous species in the lower zones, leading to reproduction.

A similar opposing seasonal preference, with more agglutinated species in winter and more calcareous taxa in summer, has previously been observed in Chezzetcook Inlet, Nova Scotia by Scott and Medioli (1980b).

No obvious changes in A/C% are found along the distance-to-sea gradient as predicted by the general trend observed in estuaries with a gradual change from agglutinated-dominated assemblages upstream to calcareous-dominated assemblages downstream (Cearreta, 1988; Debenay et al., 2000). The low number of samples collected in the middle and upper estuary, along with the inclusion of the lower estuary's upper elevation samples (rich in saltmarsh agglutinated species such as *Jadammina macrescens* and *Trochammina inflata*) weaken the correlation between A/C% and distance-to-sea. Nevertheless, in the species distribution model for each season provided by redundancy analysis (Figs. 6a and 7a), there is a clear separation between the most diverse calcareous assemblages located at the lower elevations of the lower estuary and the less diverse agglutinated assemblages located at the upper elevations of the lower estuary and middle elevations of middle and upper estuary.

Miliolids are usually reported as preferring warm, shallow (Haynes, 1981) and hypersaline waters (Douglas, 1979), as well as subtidal environments, being attached to seagrass (*Posidonia*) rhizomes (Colom, 1974; Ribes et al., 2000; Ribes and Gracia, 1991; Sen Gupta, 1999). Laboratory experiments testing the resistance of hyaline, porcelaneous and agglutinated tests to different preservation environments indicate that porcelaneous tests are very susceptible to dissolution (Camacho, 2012). Accordingly, higher densities and diversity of porcelaneous species were expected in the lower elevations during summer, where lower TOC and higher pH values are reported and both temperatures and salinities are higher, favoring calcite precipitation (Suguio, 2003). Instead, higher densities and diversities are reported in samples located in the upper elevations during winter. Comparable results, with large numbers of miliolids in relatively stable, slightly hypersaline, restricted environments were reported by Debenay et al. (1998) and Debenay and Guillou (2002). Horton and Murray (2007) found a notable number of *Quinqueloculina* spp. among typically high-marsh assemblages, and Armynot du Châtelet et al. (2009) found their most common miliolid species at different elevations along their saltmarsh transect. The latter observations, and those obtained in the present work, suggest that miliolids are somehow independent of elevation and organic matter presence, probably possessing a physiological strategy that enables them to live in such acidic environments.

5.3. Spatial and temporal environmental trends

In the present study, redundancy analysis was used to correlate several environmental variables and select the most important ones in controlling the distribution of foraminifera in winter and summer. The results agree with Murray's

(2001) model based on the niche concept, which states that local distributions are explained by a range of different factors (reaching critical thresholds singly or in combination) at different times and in different places. Indeed, the present data suggest that foraminiferal distribution reflect the seasonal variation of environmental factors and that the importance of each factor depends on the proximity of the species' ecological thresholds.

When running winter and summer models with the most important variables, considering the effects that each variable brings in addition to elevation, the majority of the variables are not significant in explaining foraminiferal distribution (see conditional effects in Table 3). Thus, elevation is the primary driver of foraminiferal assemblages as by combining the effects of a series of other environmental variables (TOC, C/N and mud content increase with increasing elevation; and pH_{sed}, sand, gravel and temperature increase with decreasing elevation).

Distance-to-sea is the second most important variable in species distribution as it controls the effects of the majority of the chemical gradients (Camacho et al., 2014). Among the chemical gradients, salinity was expected to be selected as a primer variable in species distribution. However, salinity is negatively correlated to Dsea variable and the model excludes it as primer variable due to its coliniarity.

5.4. Foraminiferal seasonal zonation

Seasonal variations in living foraminifera assemblages of the Guadiana Estuary did not significantly alter the dominant species of the various parts of the estuary. Most of these species are also dominant in transitional environments worldwide, allowing comparison between various environments in different geographical areas (Debenay and Guilou, 2002). Based on these dominant species, on their interrelationships and their relation to the environmental parameters, it was possible to define three main foraminiferal assemblages in the Guadiana Estuary:

i) The *Miliammina fusca* assemblage (Group IIb in both seasons, figs. 6 and 7): observed mainly in the mid-low elevation zones (0 to 0.75 m above MSL) of the upper reaches of the lower estuary up until the sampling northern limit, usually in unvegetated areas. *Miliammina fusca* is the dominant species (6-64%, average, 40%), associated with *Ammonia aberdoveyensis* (8-33%, average 16%) and *Elphidium oceanensis* (0-23%, average 11%);

ii) The *Jadammina macrescens* assemblage (Groups I in both seasons, figs. 6 and 7): corresponds to the most elevated, highly vegetated, marsh environments (between 1 and 2 m above MSL, or lower, in sheltered environments) of the lower estuary, where the sediments are finer (> 90% mud), pH is the lowest and TOC is the highest. *Jadammina macrescens* is the dominant species (12-100%, average 72%), with *Trochammina inflata* (0-49%, average 10%) as a subsidiary species. Occasionally, significant occurrences of *Miliammina obliqua*, *Polysaccammina ipohalina* and miliolids are recorded;

iii) The *Ammonia aberdoveyensis* assemblage (Groups IIa in both seasons, figs. 6 and 7): observed in the lower elevation zones of the lower estuary, between -0.7 to 0.3 m in relation to MSL. It corresponds to the estuarine zone of higher marine influence, where the sediment is composed of more than 50% sand, with the occasional occurrence of significant bioclastic content. *Ammonia aberdoveyensis* is the dominant species (6-61%, average 36%), associated with *Haynesina germanica* (2-41%, average 18%), *Polysaccammina hyperhalina* (0-36%, average 8%) and *Elphidium oceanensis* (0-17%, average 5%). In winter, *Bolivina ordinaria* (0-46%, average 19%) is co-dominant with *A. aberdoveyensis*.

The results achieved in the present study bring new insights in estuarine foraminiferal distribution and ecology and constitute the most complete data set yet available for the study area. Nevertheless, we believe that a higher sampling frequency could lead to a refinement of the distribution boundaries, especially in the northern portion of the estuary, where a series of interesting species occur (*Polysaccammina ipohalina*, *Miliammina obliqua*, *Haplophragmoides* spp., *Siphotrochammina* sp., etc.).

5.5. Implications to paleoenvironmental interpretations

The present results suggest that foraminiferal adaptations to multiple and inter-related parameters lead to distinct life positions of the different species. In a paleoenvironmental record, each of these life positions is more or less difficult to interpret depending on the strength of the indicative meaning of the species or assemblages present. Each species has its own unique niche influenced by a large number of abiotic and biotic factors. For a species to survive, the numerical values of all those factors must lie within the upper and lower critical threshold tolerance limits peculiar to that species (Murray, 2001; 2003). The species with a wide range of tolerance for a high number of factors have broader distributions, occupying a wide variety of habitats (eurytopic species). Conversely, species with narrow ranges of tolerance to environmental factors and consequently, narrow distributions (stenotopic species), have higher diagnostic power due to their environmental exclusivity (Odum, 1997). However, when environmental exclusivity is associated to intermittency, as is the case of *Discorinopsis aguayoi, Siphotrochammina* sp. and *Polysaccammina ipohalina* (which occur at high densities but only once in time and/or space), it is difficult to understand which precise environmental parameters governs species distribution. Among the species distributed in the Guadiana Estuary, *Jadammina macrescens, Miliammina fusca* and, to a lesser extent, *Polysaccammina hyperhalina* and *Trochammina inflata* represent the highest ecological indicator value as they are strongly related to at least one of the main environmental factors. Both, winter and summer RDAs, triplots comprising environmental, species and sample data (Fig. 6 and 7, respectively), show that *J. macrescens* is indicative of upper elevations in the lower estuary, *M. fusca* is indicative of mid-low elevations in the mid-upper estuary, *P. hyperhalina* is indicative of lower elevations in the lower estuary and *T. inflata* is indicative of middle elevations in confined environments of the lower estuary. Nevertheless, *M. fusca* and *P. hyperhalina,* which are common in the Guadiana Estuary today, are absent from the paleo-record due to higher susceptibility to taphonomic processes (Camacho, 2004). In these cases, only the signal given by the remaining paleo-assemblage can provide some answers and, together with other environmental proxies, may be used to infer the probable past ‘presence’ of the absent species. Likewise, species like *Ammonia aberdoveyensis,* which is the most ubiquitous calcareous species in the estuary presently and thus, indicative of a relatively high range of habitats, offers limited interpretative power if not considered alongside the complete assemblage, which, in this case, better reflects the prevailing environmental conditions.

The present results also indicate that, for sea-level studies where elevation is the variable of interest, the middle elevation environments are the most difficult to interpret due to their more variable nature in terms of species composition, occupying quite different positions in the RDA projection in winter and summer (e.g. FB2, LEZ2 and LG2). Their transitional position can favor species migration from the elevational extremes, promoting seasonal variability in species composition. The upper marsh zone samples (group I in Figs. 6 and 7) provide the most accurate information for sea-level reconstruction as they have a very constant species composition through time. This was earlier observed by Scott and Medioli (1980a), who, through high-resolution sampling along their Chezzetcook transects showed that the most elevated faunal zone yielded higher accuracy as it has the lowest vertical range and because the top of this zone is distinguished by a sharp decrease in foraminiferal numbers that accurately locates the highest high water (HHW) datum.

The information resulting from this study provides important ecological baselines for the interpretation of paleoenvironments and past sea-levels. Nevertheless, the taphonomic processes acting on benthic foraminifera after death and burial recorded in the paleo-record, are best analyzed using the total assemblage (living and dead individuals), including the empty tests of indigenous species, ephemeral occurrences and transported, and sometimes reworked, allochthonous species.

6. Conclusions

Foraminiferal distribution in the lower Guadiana Estuary reflects seasonal trends in environmental factors. The relative importance of these factors varies according to the proximity of species thresholds. Elevation proved to be the most important parameter controlling the distribution of foraminifera, since it combines the effect of a series of other variables, such as sedimentary organic matter and fine sediment content, which tend to increase as elevation increases, and the pH of the sediment, coarse sediment content and temperature, which tend to decrease with increasing elevation.

A negative correlation between diversity and dominance exists along the elevation gradient. In the most elevated zones, where the environmental conditions are generally harsher, only a few well adapted agglutinated species are able to survive. In the less elevated zones, where the duration of subaerial exposure is shorter and the environmental conditions are less variable, there are more diverse assemblages, mainly composed of calcareous species. In winter, when fluvial processes prevail, agglutinated species proliferate, especially those living in the uppermost zones of the marshlands. In summer, when marine conditions prevail, calcareous species become more competitive, increasing their numbers and moving further up the marsh and estuary.

Jadammina macrescens (agglutinated) and *Ammonia aberdoveyensis* (calcareous) were the most ubiquitous and abundant species in the two seasons studied.

Based on dominant species and seasonal variations in their relationships, it is possible to define a foraminiferal zonation for the Guadiana Estuary, through the distinction of three main assemblages: (i) *Miliammina fusca* assemblage, which dominates in unvegetated areas of the mid-low marsh of the mid-upper estuary; (ii) *Jadammina macrescens* assemblage, which dominates in the highest marsh areas in the lower estuary; and (iii) *Ammonia aberdoveyensis* assemblage, which dominates the areas of low marsh and tidal-flats of the lower estuary.

The upper elevation zones provide the most accurate information for sea-level reconstruction as they have the most constant species composition through time.
The data analyzed in this work bring new insights into foraminiferal distribution and are expected to improve their value as bioindicators, providing a benchmark for future environmental quality assessments and to improve the ecological interpretation of palaeoenvironmental data on the southern Iberian Peninsula and related bioclimatic zones. Future studies should concentrate on repeated sampling under different climatic conditions (e.g. dry years) to infer to what extent the patterns change, as well as with a higher sampling resolution to more accurately define the distribution boundaries of foraminiferal species, especially those living in the uppermost sector of the estuary.

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Captions:

Figure 1. Location of the study area; A) Geographical context of the Guadiana River basin in the Iberian Peninsula, Southern Europe (adapted from Confederación Hidrográfica del Guadiana (2012) in www.chguadiana.es; coordinate system: datum ETRS89 UTM Zone 30N); B) Study area: Map of Guadiana River Estuary with sampling site locations.

Figure 2. Halophytic and topographic characterization of profile E, one of the seven surveyed profiles.

Figure 3. Box-plots representing: A) faunistic density (Ni/20 cc sediment); B) number of species (S); C) Shannon diversity (Hs); and D) Agglutinated/calcareous ratio (A/C%) of the living foraminifera assemblage according to the elevational gradient (upper marsh zone: 1-2 m; lower marsh zone: 0-1 m; mud zone: -1-0 m). Median value is represented by the horizontal line within the box, 25% and 75% quartiles are defined in the ends of the box, the whiskers represent the non-outlier range and the circles represent the outliers. Extreme values are not shown.

Figure 4. Comparison between biocenotic indices measured in winter and summer according to the elevational gradient (upper marsh zone: 1-2 m; lower marsh zone: 0-1 m; mud zone: -1-0 m): A – faunistic density (Ni); B – number of species (S); C – Shannon diversity (Hs); D – Agglutinated/calcareous ratio (A/C%). The vertical axis represents distance to sea variable. Only Ni and S were analyzed and described for all 49 samples in winter and summer. For calculating the remaining metrics, only statistically valid samples were considered.

Figure 5. Variation of agglutinated, hyaline and porcelaneous tests percentage in the statistical valid samples collected along distance to sea and elevation gradients in winter and summer.

Figure 6. Winter distribution model of benthic living foraminifera: A) RDA triplot summarizing the effects of the main driving environmental variables (Elev and Dsea)

and B) Shade matrix for the main 18 living species (abundance > 5%) and 19 samples along the estuary.

Figure 7. Summer distribution model of benthic living foraminifera: A) RDA triplot summarizing the effects of the main driving environmental variables (Elev, Dsea and Gmean) and B) Shade matrix for the main 15 living species (abundance > 5%) and 23 samples along the estuary.

Plate 1 – Scanning electron micrographs of the foraminifera specimens. Scale bar - 100 µm except fig. 3 with scale bar – 10 µm. 1-3) *Jadammina macrescens*; 1) dorsal view; 2) view of the supplementary apertures; 3) detail view of the supplementary apertures; 4-5) *Trochammina inflata*; 4) dorsal view; 5) ventral view; 6-7) *Siphotrochammina* sp.; 6) dorsal view, with a inter-cameral foramen; 7) ventral view; 8) *Polysaccammina hyperhalina*; 9) *Polysaccammina ipohalina*; 10) *Miliammina obliqua*, with view of the interio-marginal arch of the aperture; 11) *Miliammina fusca*; 12-14) Miliolid sp3; 12) front view; 13) apertural view; 14) back view; 15) *Bolivina ordinaria*; 16-17) *Discorinopsis aguayoi*; 16) dorsal view; 17) ventral view; 18) *Haynesina germanica*; 19) *Elphidium oceanensis*; 20-22) *Ammonia aberdoveyensis* (sp1 variant); 20) dorsal view; 21) profile view; 22) ventral view; 23-24) *Asterigerinata mamilla*; 23) dorsal view; 24) ventral view.

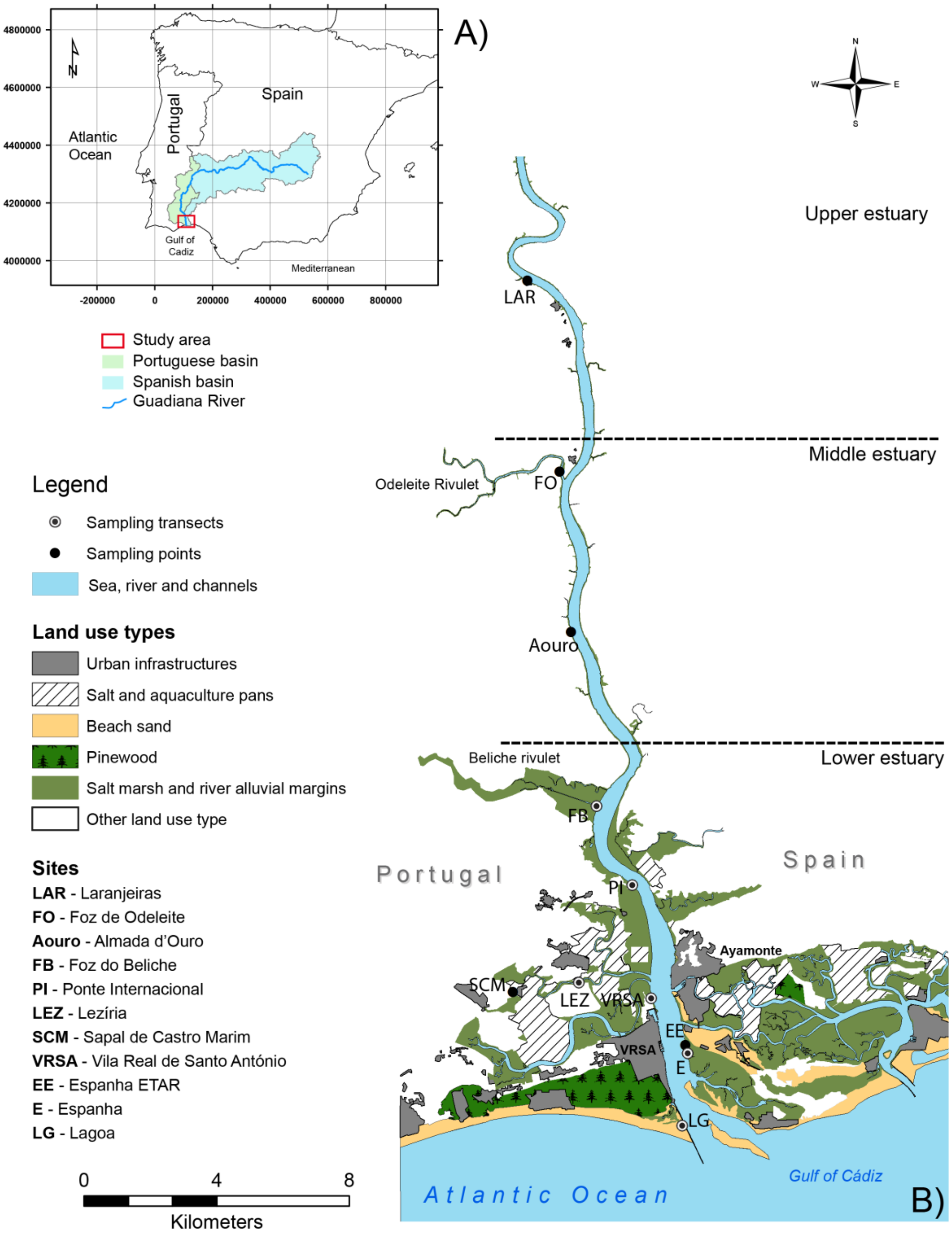

A)
4800000
4600000
4400000
4200000
4000000
-200000
0
200000
400000
600000
800000
N
Atlantic Ocean
Portugal
Spain
Gulf of Cadiz
Mediterranean
Study area
Portuguese basin
Spanish basin
Guadiana River
N
W
E
S
Upper estuary
LAR
Middle estuary
Odeleite Rivulet
FO
Aouro
Beliche rivulet
Lower estuary
FB
Portugal
Spain
PI
Ayamonte
SCM
LEZ
VRSA
EE
VRSA
E
LG
Gulf of Cádiz
Atlantic Ocean
B)
Legend
Sampling transects
Sampling points
Sea, river and channels
Land use types
Urban infrastructures
Salt and aquaculture pans
Beach sand
Pinewood
Salt marsh and river alluvial margins
Other land use type
Sites
LAR - Laranjeiras
FO - Foz de Odeleite
Aouro - Almada d'Ouro
FB - Foz do Beliche
PI - Ponte Internacional
LEZ - Lezíria
SCM - Sapal de Castro Marim
VRSA - Vila Real de Santo António
EE - Espanha ETAR
E - Espanha
LG - Lagoa
0
4
8
Kilometers

Figure 1

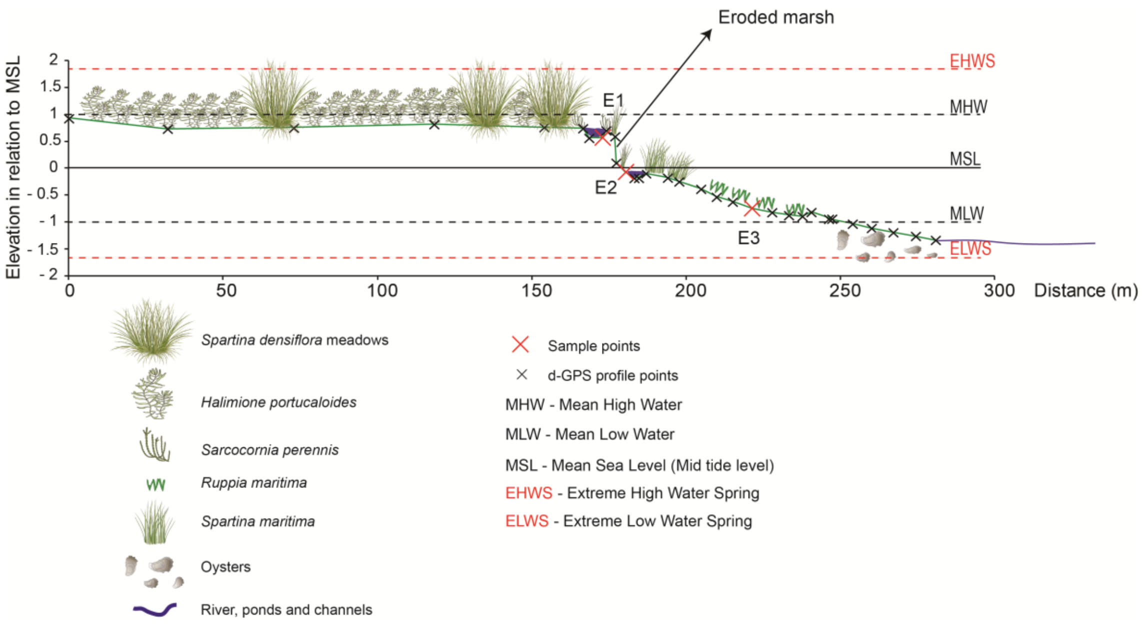

Eroded marsh
Elevation in relation to MSL
2
1.5
1
0.5
0
- 0.5
- 1
- 1.5
- 2
EHWS
MHW
MSL
MLW
ELWS
E1
E2
E3
0
50
100
150
200
250
300
Distance (m)
Spartina densiflora meadows
Halimione portucaloides
Sarcocornia perennis
Ruppia maritima
Spartina maritima
Oysters
River, ponds and channels
Sample points
d-GPS profile points
MHW - Mean High Water
MLW - Mean Low Water
MSL - Mean Sea Level (Mid tide level)
EHWS - Extreme High Water Spring
ELWS - Extreme Low Water Spring

Figure 2

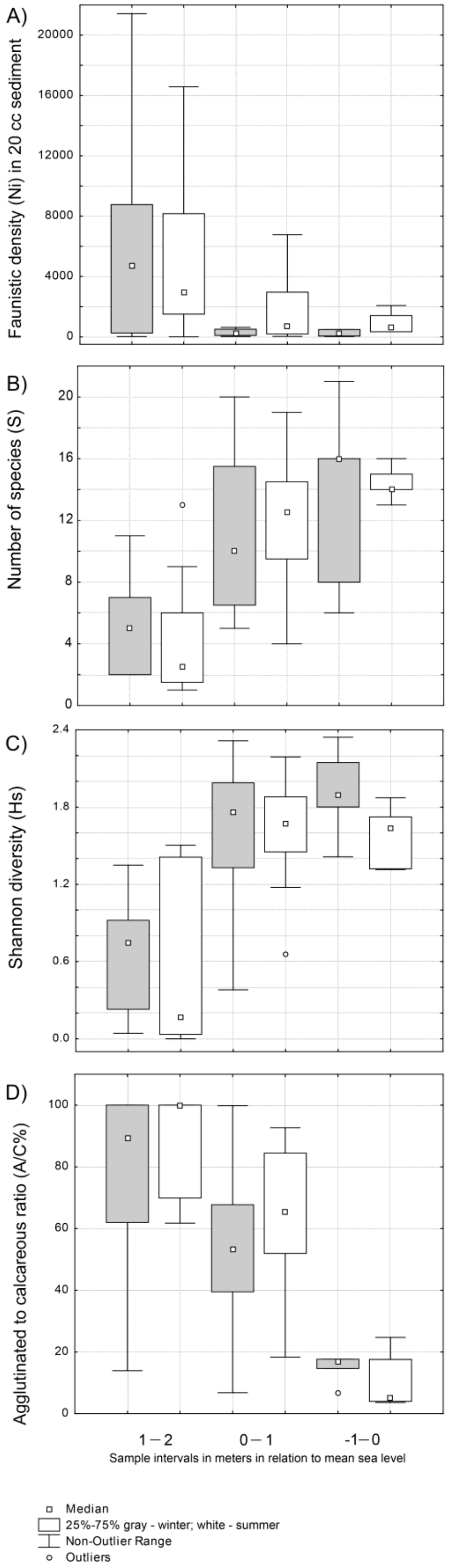
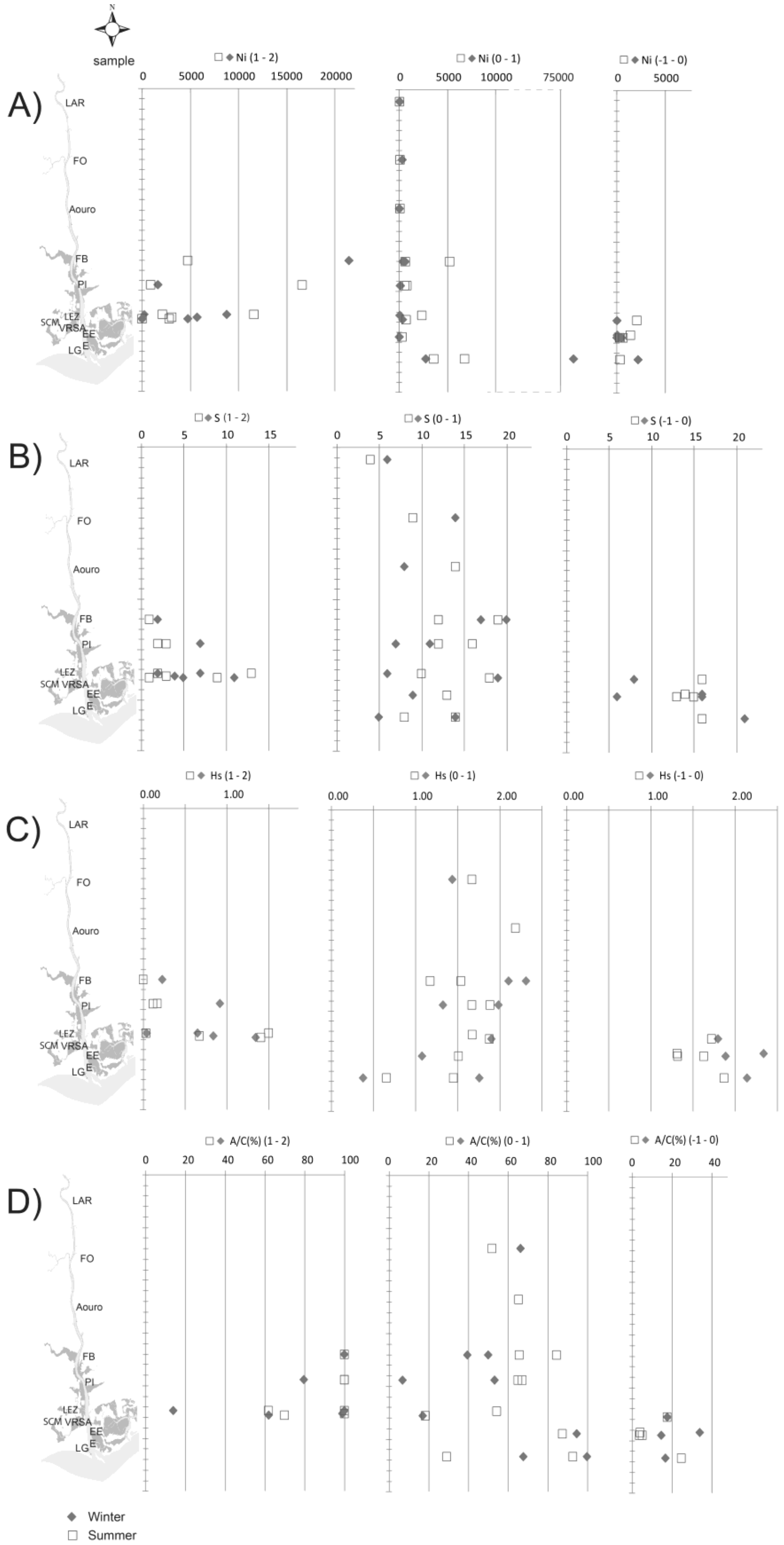

A)
Faunistic density (Ni) in 20 cc sediment
0
4000
8000
12000
16000
20000
B)
Number of species (S)
0
4
8
12
16
20
C)
Shannon diversity (Hs)
0.0
0.6
1.2
1.8
2.4
D)
Agglutinated to calcareous ratio (A/C%)
0
20
40
60
80
100
1 – 2
0 – 1
-1 – 0
Sample intervals in meters in relation to mean sea level
Median
25%-75% gray - winter; white - summer
Non-Outlier Range
Outliers

Figure 3



Ni - faunistic density (individuals/20cc)
S - number of species
Hs - Shannon diversity
A/C% - Agglutinated/calcareous ratio

Figure 4

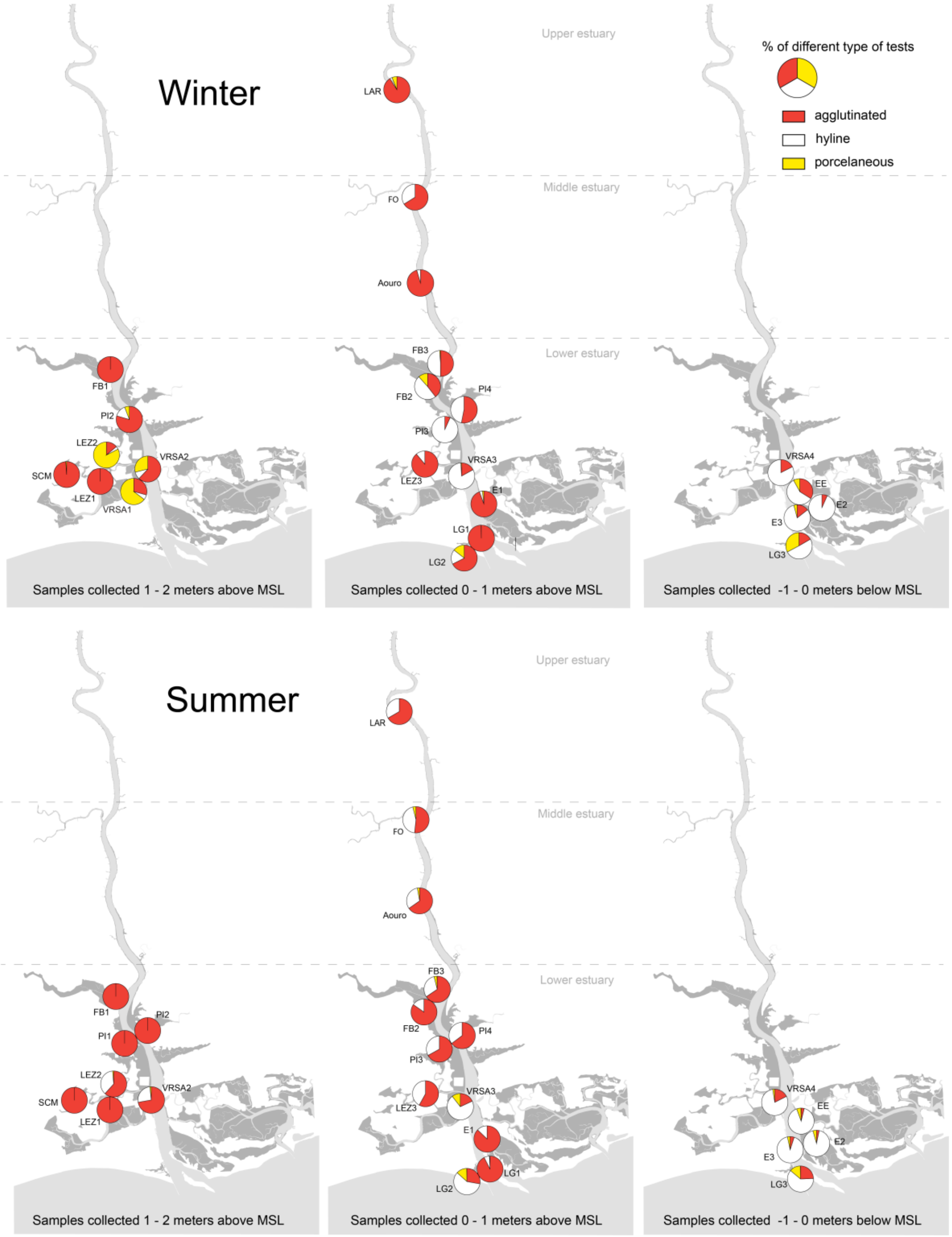

Winter
Upper estuary
LAR
% of different type of tests
🟥 agglutinated
☐ hyline
🟨 porcelaneous
Middle estuary
FO
Aouro
Lower estuary
FB1
PI2
LEZ2
VRSA2
SCM
LEZ1
VRSA1
FB3
FB2
PI4
PI3
VRSA3
LEZ3
E1
LG1
LG2
VRSA4
EE
E2
E3
LG3
Samples collected 1 - 2 meters above MSL
Samples collected 0 - 1 meters above MSL
Samples collected -1 - 0 meters below MSL
Summer
Upper estuary
LAR
Middle estuary
FO
Aouro
Lower estuary
FB1
PI2
PI1
LEZ2
SCM
VRSA2
LEZ1
FB3
FB2
PI4
PI3
LEZ3
VRSA3
E1
LG1
LG2
VRSA4
EE
E2
E3
LG3
Samples collected 1 - 2 meters above MSL
Samples collected 0 - 1 meters above MSL
Samples collected -1 - 0 meters below MSL

Figure 5

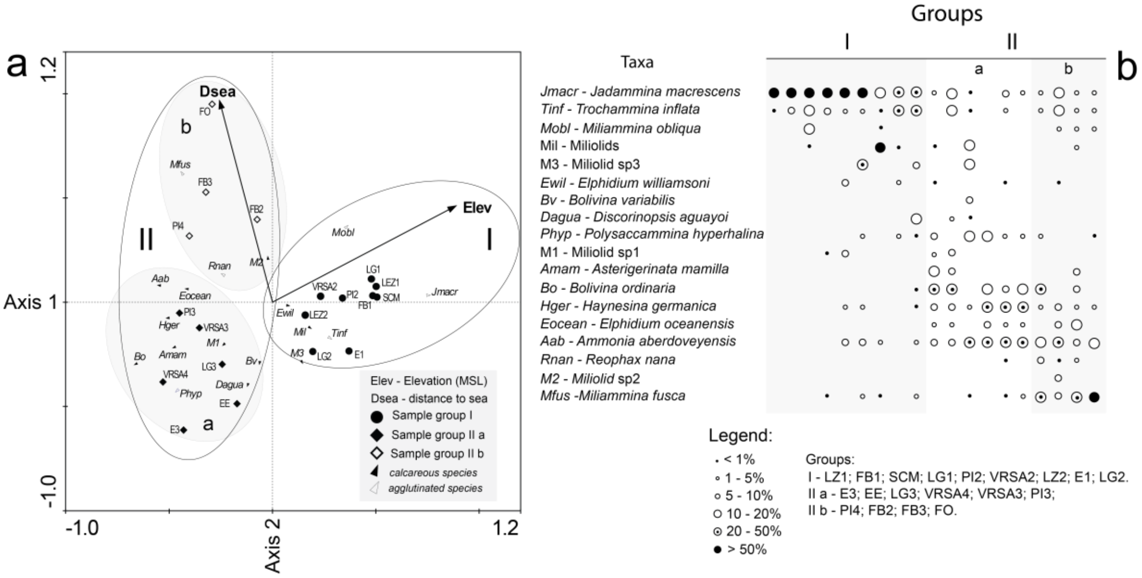
a
1.2
Dsea
FO
b
Mfus
FB3
FB2
PI4
II
Mobl
Elev
I
Rnan
M2
LG1
LEZ1
Aab
VRSA2
PI2
Jmacr
SCM
FB1
Axis 1
Eocean
Ewil
LEZ2
PI3
Hger
VRSA3
Mil
Tinf
M1
Bo
Amam
M3
LG2
E1
LG3
Bv
VRSA4
Dagua
Phyp
EE
E3
a
Elev - Elevation (MSL)
Dsea - distance to sea
Sample group I
Sample group II a
Sample group II b
calcareous species
agglutinated species
-1.0
-1.0
Axis 2
1.2
Groups
I
II
Taxa
a
b
b
Jmacr - Jadammina macrescens
Tinf - Trochammina inflata
Mobl - Miliammina obliqua
Mil - Miliolids
M3 - Miliolid sp3
Ewil - Elphidium williamsoni
Bv - Bolivina variabilis
Dagua - Discorinopsis aguayoi
Phyp - Polysaccammina hyperhalina
M1 - Miliolid sp1
Amam - Asterigerinata mamilla
Bo - Bolivina ordinaria
Hger - Haynesina germanica
Eocean - Elphidium oceanensis
Aab - Ammonia aberdoveyensis
Rnan - Reophax nana
M2 - Miliolid sp2
Mfus -Miliammina fusca
Legend:
< 1%
1 - 5%
5 - 10%
10 - 20%
20 - 50%
> 50%
Groups:
I - LZ1; FB1; SCM; LG1; PI2; VRSA2; LZ2; E1; LG2.
II a - E3; EE; LG3; VRSA4; VRSA3; PI3;
II b - PI4; FB2; FB3; FO.

Figure 6

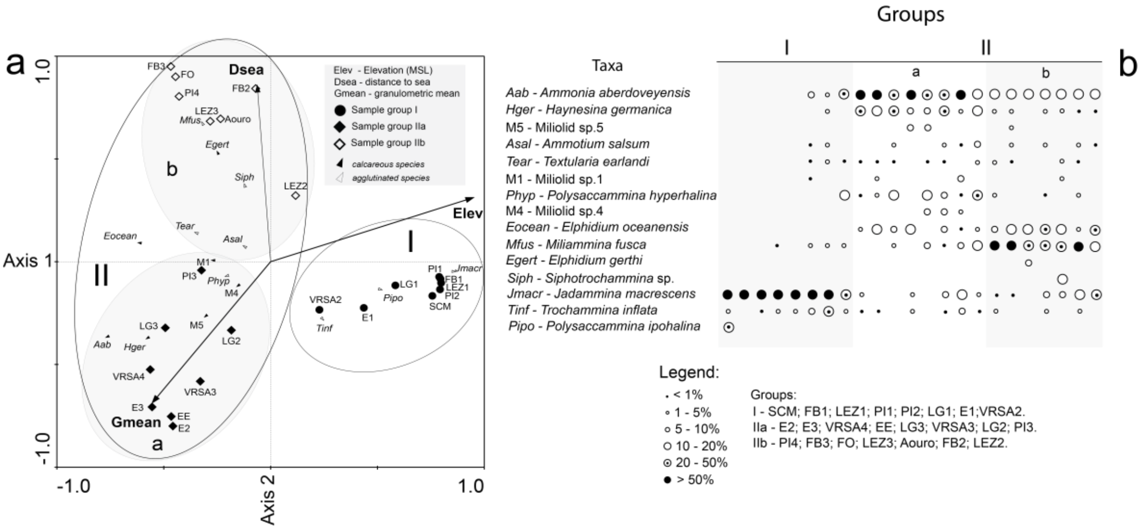

a
b
1.0
-1.0
Axis 1
Axis 2
Dsea
Elev
Gmean
I
II
a
b
Elev - Elevation (MSL)
Dsea - distance to sea
Gmean - granulometric mean
● Sample group I
◆ Sample group IIa
◇ Sample group IIb
calcareous species
agglutinated species
FB3
FO
PI4
FB2
LEZ3
Aouro
Mfus
Egert
Siph
LEZ2
Eocean
Tear
Asal
M1
PI3
Phyp
M4
M5
LG3
LG2
Aab
Hger
VRSA4
VRSA3
E3
EE
E2
VRSA2
Tinf
E1
Pipo
LG1
PI1
Jmacr
FB1
LEZ1
PI2
SCM
Groups
Taxa
Aab - Ammonia aberdoveyensis
Hger - Haynesina germanica
M5 - Miliolid sp.5
Asal - Ammotium salsum
Tear - Textularia earlandi
M1 - Miliolid sp.1
Phyp - Polysaccammina hyperhalina
M4 - Miliolid sp.4
Eocean - Elphidium oceanensis
Mfus - Miliammina fusca
Egert - Elphidium gerthi
Siph - Siphotrochammina sp.
Jmacr - Jadammina macrescens
Tinf - Trochammina inflata
Pipo - Polysaccammina ipohalina
Legend:
< 1%
1 - 5%
5 - 10%
10 - 20%
20 - 50%
> 50%
Groups:
I - SCM; FB1; LEZ1; PI1; PI2; LG1; E1;VRSA2.
IIa - E2; E3; VRSA4; EE; LG3; VRSA3; LG2; PI3.
IIb - PI4; FB3; FO; LEZ3; Aouro; FB2; LEZ2.

Figure 7

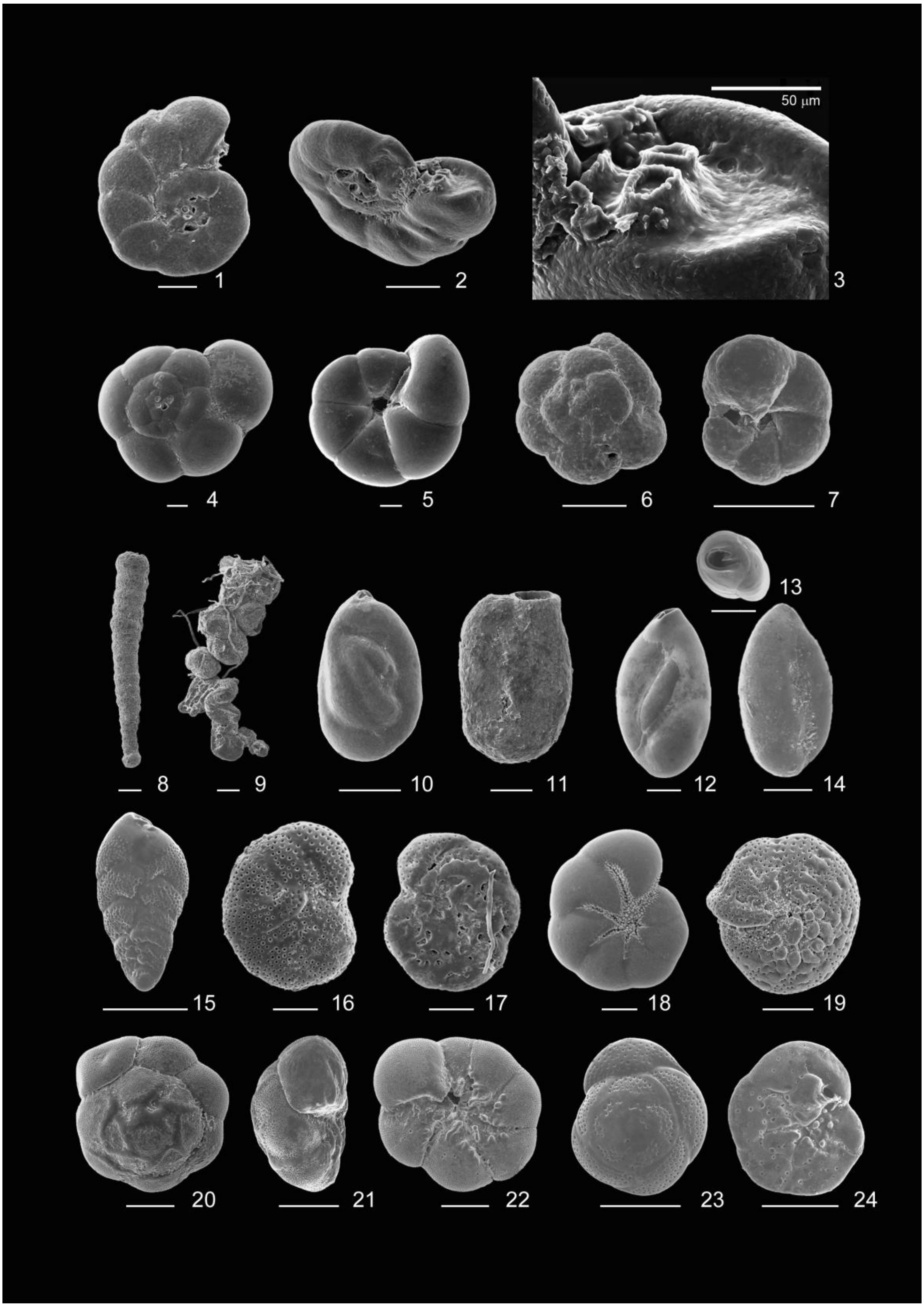


Plate I

Table 1 – Spearman's Rank Correlation (*R*) or **Pearson Correlation Coefficient (*r*)** between spatial and physic-chemical variables. According to the normality of the variable, (*R*) or **(*r*)** is indicated for each pairwise, and are significant for $p < 0.01$ (**) and for $p < 0.05$ (*).

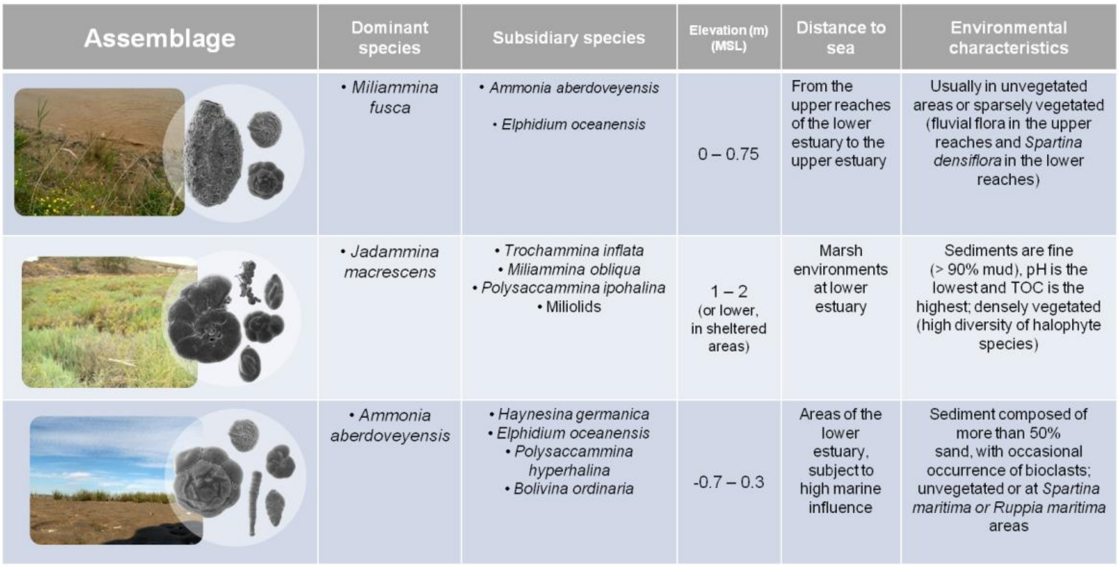
Season	winter				summer			
parameters	Ni	S	Hs	A/C%	Ni	S	Hs	A/C%
Elev	0.5*	**-0.61****	**-0.7****	0.51*	0.38	-0.68**	-0.51*	0.9**
Dsea	-0.18	-0.13	-0.04	0.06	-0.07	-0.2	0.05	0.36
Ni/20cc	-	-0.35	-0.51*	0.61**	-	-0.52*	-0.72**	0.46*
S	-0.35	-	**0.89****	-0.55*	-0.52*	-	0.71**	-0.7**
Hs	-0.51*	**0.89****	-	-0.57*	-0.72**	0.71**	-	-0.57**
A/C%	0.61**	-0.55*	-0.57*	-	0.46*	-0.7**	-0.57**	-

Table 2 – Synthesis of the biocenotic parameters (Ni – faunistic density – nº individuals/20 cc of sediment; S – nº of species/20 cc of sediment; Hs – Shannon diversity; A/C% - agglutinated/calcareous tests ratio) for the samples of winter and summer according to an elevational gradient (upper marsh zone: 1-2 m; lower marsh zone: 0-1 m; mud zone: -1-0 m).

Season	winter			summer		
Elev MSL (m)	1 - 2	0 - 1	-1 - 0	1 - 2	0 - 1	-1 - 0
Ni/20cc						
max	21424	76224	2200	16576	6768	2072
min	17	24	15	2	31	332
mean	6079	6770	591	5224	1756	956
SD	7472	21884	918	5803	2263	763
S						
max	11	20	21	13	19	16
min	2	5	6	1	4	13
mean	5	11	13	4	12	14
SD	3	5	6	4	4	1
Hs						
max	1.349	2.315	2.343	1.50	2.19	1.87
min	0.043	0.380	1.801	0.00	0.66	1.31
mean	0.673	1.589	2.045	0.56	1.57	1.57
SD	0.478	0.601	0.246	0.65	0.40	0.25
A/C%						
max	100	99.83	34	100	92.67	24.70
min	13.92	6.78	14.63	61.74	18.31	3.61
mean	75.75	55	20.75	90.23	61.93	10.98
SD	33.9	31.45	8.92	16.84	23.03	4.37

Table 3 – Marginal and conditional effects and significance of the main variables in foraminifera distribution for each season, obtained from the summary of forward selection in RDA. For more details in environmental variables statistical selection see online supplementary material.

	Variable	*Marginal effects (λ1)*	*Conditional effects (λA)*	***p***
winter	*Elev*	*0.24*	*0.24*	***0.004***
	TOC	*0.23*	*0.07*	*0.064*
	sand	*0.15*	*0.02*	*0.730*
	mud	*0.15*	*0.02*	*0.730*
	T	*0.11*	*0.05*	*0.174*
	Dsea	*0.09*	*0.12*	***0.006***
	DO	*0.09*	*0.04*	*0.288*
summer	*Elev*	*0.39*	*0.39*	***0.002***
	sand	*0.30*	*0.04*	*0.164*
	mud	*0.30*	*0.04*	*0.164*
	Gmean	*0.19*	*0.05*	***0.024***
	DO	*0.18*	*0.02*	*0.286*
	T	*0.16*	*0.02*	*0.584*
	pHsed	*0.11*	*0.03*	*0.330*
	Dsea	*0.10*	*0.13*	***0.002***



Assemblage	Dominant species	Subsidiary species	Elevation (m) (MSL)	Distance to sea	Environmental characteristics
	• *Miliammina fusca*	• *Ammonia aberdoveyensis* • *Elphidium oceanensis*	0 – 0.75	From the upper reaches of the lower estuary to the upper estuary	Usually in unvegetated areas or sparsely vegetated (fluvial flora in the upper reaches and *Spartina densiflora* in the lower reaches)
	• *Jadammina macrescens*	• *Trochammina inflata* • *Miliammina obliqua* • *Polysaccammina ipohalina* • Miliolids	1 – 2 (or lower, in sheltered areas)	Marsh environments at lower estuary	Sediments are fine (> 90% mud), pH is the lowest and TOC is the highest; densely vegetated (high diversity of halophyte species)
	• *Ammonia aberdoveyensis*	• *Haynesina germanica* • *Elphidium oceanensis* • *Polysaccammina hyperhalina* • *Bolivina ordinaria*	-0.7 – 0.3	Areas of the lower estuary, subject to high marine influence	Sediment composed of more than 50% sand, with occasional occurrence of bioclasts; unvegetated or at *Spartina maritima or Ruppia maritima* areas

Graphical abstract