DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

Intertidal meiofauna communities along the south arm of Mondego estuary: Spatial and temporal variability in relation to environmental parameters

Conceição Alexandra Costa Caetano

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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 João Carlos Marques (Universidade de Coimbra) e do Doutor José Lino Costa (Universidade de Lisboa)

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ABSTRACT

Meiobenthic communities exhibit heterogeneous spatial distributions and seasonal variations due to both natural and anthropogenic driven pressures. The main goal of this study was to investigate the spatial and temporal patterns of the intertidal meiobenthic assemblages of a Portuguese temperate mesotidal estuary and relate them with the environment. Benthic samples were collected along the southern branch of the Mondego River estuary in three sampling occasions at four locations, and several environmental parameters were measured simultaneously. Both taxonomic and functional approaches were applied to meiofauna in general and also more specifically to nematodes (usually the dominant group in meiofauna) communities to describe their structure relating it to environmental parameters along the south arm of the Mondego estuary. Seasonal heterogeneity appeared to be more important than spatial heterogeneity in differentiating both communities, with higher values of density and diversity occurring in autumn. The meiofauna communities showed a dominance of Nematoda (as expected), followed by Copepoda and Polychaeta. The Nematoda genera identified resembled those of the Northern European estuaries. The distribution patterns of meiofauna in general and nematodes were commonly structured by three main environmental factors, namely phosphates concentration, salinity and dissolved oxygen.

This study suggests that information based on meiofauna higher taxa resolution may provide a sensitive and clear measure of estuarine environmental condition, as much as nematode genera.

RESUMO

As comunidades meiobentónicas apresentam distribuições espacialmente heterogéneas e experienciam variações sazonais devido a pressões naturais e antropogénicas. O principal objetivo deste estudo foi investigar os padrões espaciais e temporais das comunidades de meiobentos na zona intertidal de um estuário temperado mesotidal e identificar as variáveis ambientais mais importantes que influenciam estas comunidades. Nesse sentido, foram recolhidas amostras de material bentónico em quatro locais ao longo do ramo sul do estuário do rio Mondego em três períodos diferentes. Simultaneamente foram medidos vários parâmetros ambientais.

Para descrever a estrutura das comunidades de meiofauna (em geral) e mais especificamente dos nematodes (geralmente o grupo dominante da meiofauna) e relacioná-las com os parâmetros ambientais, foram aplicadas tanto a abordagem taxonómica como funcional. A variabilidade sazonal pareceu ser mais importante do que a heterogeneidade espacial na diferenciação entre as duas comunidades, verificando-se valores mais elevados de densidade e diversidade durante o outono. As comunidades de meiofauna mostraram um predomínio de Nematoda (como esperado), seguido dos taxa Copepoda e Polychaeta. Os géneros de nemátodes identificados assemelharam-se aos descritos para os estuários do norte europeu. Os padrões de distribuição de meiofauna em geral e nemátodes em particular foram influenciados por três principais fatores ambientais, nomeadamente a concentração de fosfatos, a salinidade e o oxigênio dissolvido.

Este estudo sugere que tanto os nemátodes (menor resolução taxonómica) como a meiofauna em geral (maior resolução taxonómica) podem fornecer uma medida sensível e clara da condição ambiental estuarina.

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1. INTRODUCTION

1. INTRODUCTION

Estuarine areas are among the most productive ecosystems (Kennish, 2002; Dolbeth et al., 2003). Their importance is recognised worldwide, for providing essential ecological functions (decomposition, nutrient cycling and flux regulation of water, particles and pollutants) and services, such as habitat, protection, food for migratory and resident species, shoreline protection, fisheries resources, navigation routes and harbours, and recreational purposes (Kennish, 2002; Paerl, 2006).

Estuaries provide a natural gradient of salinity, often closely linked to other estuarine gradients, where abiotic conditions can change appreciably and continuously over a scale of kilometres. The natural gradient of salinity, linked with others (e.g. bed sediment type and dynamics, oxygen availability, temperature and current speed), are well documented as important factors in determining temporal and spatial variations of benthic communities (Bouwman, 1983; Heip et al., 1985; Austen & Warwick, 1989; Soetaert et al., 1995; Li et al., 1997; Forster, 1998; Moens & Vincx, 2000; Steyaert et al., 2003; Derycke et al., 2007; Alves et al., 2009; Adão et al., 2009; Patrício et al., 2012; Alves et al., 2013). This physicochemical gradient is dependent on the size and shape of the estuary, and therefore a single water body can show different physicochemical conditions throughout its extension (Bald et al., 2005).

Climate variations can also have important impacts on aquatic ecosystems (Roessig et al., 2004), affecting their composition, structure, function, biodiversity and productivity. Extreme climatic events, such as floods or droughts are increasing in frequency worldwide (Mirza, 2003), and as a consequence, river discharge into many estuaries may be affected (Gleick,

2003, Pillay & Perissinotto, 2009). It is known that the intensification of extreme flooding events can result in catastrophic deposition of fine sediments with profound influences on the structure and function of benthic communities (Norkko et al., 2002; Salen-Picard et al., 2003), and beyond this, stochastic events can eliminate parts of populations (Scheffer et al., 2001).

As transitional areas between land and sea, most estuaries are under increasing pressure from a variety of stressors including urban, agricultural and industrial effluents, dredging activities, infrastructure construction and maritime traffic (Paerl, 1996). These anthropogenic pressures modify habitat and hydrological regimes lowering the overall system stability (Cardoso et al., 2005; Dolbeth et al., 2007), and associated with natural stressors may interact to produce combined impacts on biodiversity and ecosystem functioning.

Therefore, the estuarine community structure can change as a *continuum* on various spatial and temporal scales in relation to both natural and anthropogenic gradients (Pearson & Rosenberg, 1978; Rakocinski et al., 1997). In fact, in addition to spatial patterns, temperate estuarine communities also show important temporal variations related to seasonal and inter-annual changes. Seasonal fluctuations in abundance and composition can be due to recruitment pulses, and also to the occurrence of extreme environmental conditions (Alden et al., 1997; Atrill & Power, 2000)

Meiofauna represent an important component of estuarine benthic communities, providing ecosystem services including sediment bioturbation and recycling of organic matter (Higgins & Thiel, 1988; Nozais et al., 2005). Furthermore, meiofauna have an important role in marine benthic food chains (Heip et al., 1985; Moens et al., 2005). They estabish an important link between

primary producers, as they are consumers of microphytobenthos (Pace & Carman, 1996; Pinckney et al., 2003), and comprise an important food source to macroinvertebrate and juvenile fish that use intertidal habitats as a nursery ground (Nakagami et al., 2000; Reichert, 2003). On the other hand, due to their ecological characteristics (small size, high abundance, rapid generation times and absence of a planktonic phase) and intimate association with the sediments, meiofauna is rapidly affected by changes in abiotic and biotic environmental parameters. The subsequent changes in community structure can directly affect higher trophic organisms which depend on the meiofauna as a source of food. Therefore, meiofauna features are a good indicator of environmental conditions and changes in their density, diversity, structure and functioning may indicate alterations in the system.

Apart from natural stressors, physical and chemical anthropogenic pressures can also modify the meiofauna distribution patterns. By altering the relative abundances of sensitive species, as well as their diversity and distribution patterns, anthropogenic pressures can be key factors influencing the structure and composition of meiobenthos communities (Essink & Keidel, 1998; Schratzberger & Warwick, 1998; Schratzberger et al., 2004; Derycke et al., 2007). Therefore, characterizing the distribution patterns of meiobenthic assemblages has become a useful biological tool to detect anthropogenic disturbance and environmental change (Warwick, 1981; Coull & Chandler, 1992). Their characteristics give meiofauna several advantages over the commonly used macrofauna communities as monitoring organisms (Kennedy & Jacoby, 1999; Schratzberger et al., 2000; Austen & Widdicombe, 2006). In fact, nematodes have been pointed out as potential indicators of anthropogenic

disturbance in aquatic ecosystems (e.g. Coull &Chandler, 1992; Schratzberger et al., 2004; Steyaert et al., 2007; Moreno et al., 2008). The inclusion of information regarding their functional traits (e.g. trophic structure, life strategy) can provide critical information on the functioning of ecosystems (Norling et al., 2007; Danovaro et al., 2008).

The meiofauna communities are reasonably well characterized around the world, with studies ranging from the deep-sea floor to alpine lakes or from tropical reefs to polar sea ice (Giere, 2009). In Europe, studies on meiobenthic estuarine communities mostly include the more northerly estuarine ecosystems (e.g. Warwick & Gee, 1984; Heip et al., 1985; Li & Vincx, 1993; Smol et al., 1994; Soetaert et al., 1995; Steyaert et al., 2003; Ferrero et al., 2008; Rzeznik-Orignac et al., 2003). In southern Europe, particularly in the Iberian Peninsula, there is a notorious lack of information on both spatial and temporal distribution of meiofauna and free-living nematodes in estuarine environments. The studies on meiofauna composition and structure conducted in Portugal are limited to a comparative study between five European estuaries (Ems and Westerschelde – Netherlands; Somme and Gironde – France, and Tagus – Portugal, Soetaert et al., 1995); a PhD thesis focusing on the dynamics of intertidal meiofauna communities associated to *Zostera noltii* seagrass beds in the Mira estuary (Adão, 2000, 2003) and several studies carried out at the Mondego estuary (Adão et al., 2009; Alves et al., 2009; Patrício et al., 2012; Alves et al., 2013), all focusing on subtidal meiofauna assemblages.

The present study aimed to investigate spatial and temporal changes in the patterns of meiofauna and nematode assemblage composition, density and diversity, including some nematode functional traits, between four intertidal sampling locations localized in the south arm of the Mondego estuary among three sampling occasions, and thus to contribute to fulfil the gap of knowledge about Portuguese meiofauna communities.

The following specific questions were addressed:

(a) How meiofauna and nematode communities' structure change along the south arm subsystem of the Mondego estuary across different seasonal sampling events?

(b) What are the main natural environmental variables influencing the structure and distribution of meiofauna and nematode assemblages?

(c) Do meiofauna in general and nematode communities provide similar ecological assessment information about the south arm subsystem of the Mondego estuary?

2. MATERIAL AND METHODS

2. MATERIAL AND METHODS

2.1. Study site

The Mondego estuary (Fig. 1) is a relatively small warm-temperate polyhaline intertidal system (with 21 km long and with 860 ha of surface area) located on the NW coast of Portugal. The last 7 km, near the mouth, consist of two arms, with very different hydrological characteristics, separated by an alluvium-formed island, Murraceira. This study focused in the south arm, which is shallower (2–4 m during high tide), presenting large areas of intertidal mudflats (almost 75% of the area) exposed during low tide (Neto et al., 2008), where seagrass (*Zostera noltii*) meadows are present in some locations. Until recently, the southern upstream connection with the main river course (north arm) was totally (1994-1998) or partially interrupted (1998-2006), which had negative environmental impacts, namely regarding eutrophication effects. A full re-establishment of the communication between the two arms was undertaken during the spring of 2006, in order to improve the water quality in the terminal part of the estuary by reducing the residence time in the southern arm (Neto et al., 2010). The south arm has been considered to be the richest area of the estuary in terms of productivity and biodiversity (Marques et al., 1993), but is also under considerable human induced environmental pressures, namely nutrient-loadings coming from agriculture (mainly corn and rice fields), salt-extraction, and aquaculture farms located on Murraceira island (Marques et al., 2003).

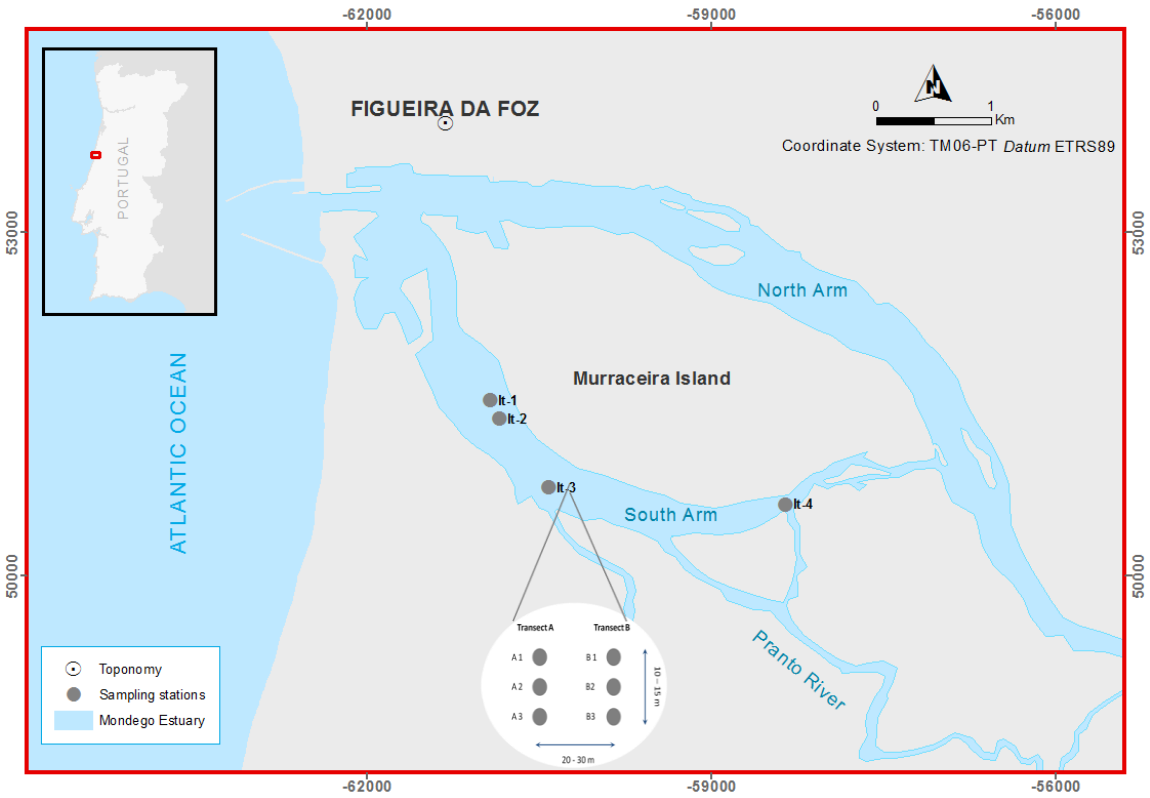

Fig. 1 - Location of sampling stations within the Mondego estuary, with detail of sampling strategy.

In scope of a regular monitoring survey of the Mondego estuary (since the nineties onwards), four sampling stations were selected in the south arm (Fig. 1): **It-1**, in an area occupied by *Zostera noltii* beds; this area is characterized by muddy sediments with high organic matter content, higher salinity values, lower total inorganic nitrogen concentrations and higher water flow velocity; **It-2,** an intermediate area, located just upstream It-1, with environmental conditions similar to the aforementioned station but with lower sediment organic matter content; **It-3,** a bare bottom area in the inner part of the subsystem, characterized by the absence of rooted macrophytes and covered occasionally by green macroalgae of genus *Ulva*; this sand flat presents lower organic matter content, lower salinities, higher total inorganic nitrogen concentrations, and lower water flow; **It-4**, a bare bottom area, located next to the upstream

communication between the two arms and adjacent to a *Scirpus maritimus* marsh, characterized by very low depths and strong freshwater influence, having the lowest salinity values.

2.2. Sampling strategy

The intertidal soft-bottom meiobenthic assemblages were sampled at each of the selected sampling stations (It-1, It-2, It-3 and It-4) (Fig. 1), in three occasions: mid-September 2009 (summer, Su-09), mid-December 2009 (autumn, Au-09) and early March 2010 (winter, Wi-10). All samples were collected between 2h before and 2h after the maximum low tide, in order to analyze the spatiotemporal variability of the physicochemical environment and meiobenthic assemblages in the south arm.

2.3. Data collection

2.3.1. Environmental data

At each sampling station, bottom water parameters were measured *in situ* with a YSI Data Sonde Survey 4: salinity (Practical Salinity Scale), temperature (°C), pH, and dissolved oxygen (DO) (mg/L). Additionally, water samples were collected for determination of nutrients in laboratory: nitrate ($N\text{-}NO_3^-$) (µmol/L) and nitrite ($N\text{-}NO_2^-$) (µmol/L) concentrations were analyzed according to standard methods as described in Strickland & Parsons (1972) and ammonium ($N\text{–}NH_4^+$) (µmol/L) and phosphate ($P\text{–}PO_4^{3-}$) (µmol/L) concentrations were analyzed following the Limnologisk Metodik (1992). Chlorophyll-a (Chl-a) determinations were performed according to Parsons et al. (1985).

Sediment samples were taken at each station to determine the organic matter content and grain size. Sediment organic matter (OM) content was

defined as the difference between the weight of each sample after oven-drying at 60 ˚C for 72 h followed by combustion at 450 ˚C for 8 h, and was expressed as the percentage of the total sample weight. Grain size was carried out by dry mechanical separation through a column of sieves of different mesh sizes, corresponding to the five classes described by Brown & McLachlan (1990): (a) gravel (>2 mm), (b) coarse sand (0.500–2.000 mm), (c) mean sand (0.250–0.500 mm), (d) fine sand (0.063–0.250 mm), and (e) silt and clay (<0.063 mm). The relative content of the different grain-size fractions was expressed as a percentage of the total sample weight.

2.3.2. Biological data

In order to evaluate the broadly recognized patchy distribution in meiofauna assemblages (e.g. Findlay, 1981, Heip et al., 1985, Schratzeberger et al., 2008) in south arm of the Mondego estuary, six replicate samples were collected at each sampling station along two transects: three samples were collected in transect A, covering a range of 10-15 m, and the other three were collected in transect B, apart from transect A for about 20 to 30 m of distance (Fig. 1). The replicate samples were collected by forcing a “Kajak” sediment corer (inner diameter: 3.6 cm) 3 cm into the sediment. All samples were preserved in a 4% buffered formalin solution and were washed through a series of nested sieves of 1mm and 38μm mesh size (Heip et al., 1985). The material retained on the smaller mesh size was collected and the meiofauna was extracted from the sediment fraction using Ludox HS-40 colloidal silica at a specific gravity of 1.18 $g.cm^{-3}$ (Vincx, 1996). All meiobenthic organisms were counted and identified at a higher taxonomic level under a stereomicroscope (magnification 40×). Meiofauna taxa identification was based on Higgins & Thiel (1988) and Giere

(2009). The density (individuals per 10 cm^2) of each taxon was quantified. A random set of 120 nematodes, or the total content of individuals in samples with less than 120 nematodes, was picked from each replicate, cleared in glycerol–ethanol solution, transferred to anhydrous glycerol by evaporation, and mounted on slides for identification (Vincx, 1996). All nematodes were identified to genus level using a microscope fitted with a 100× oil immersion objective and based on the pictorial keys of Platt & Warwick (1983, 1988), Warwick et al. (1998), and the online information system NeMys (Steyaert et al., 2005).

2.4. Data analysis

Univariate and multivariate analysis of environmental and biological data *per si* were performed using the PRIMER v6 software package (Clarke & Warwick, 2001) with the PERMANOVA add-on package (Anderson et al., 2008) and for the analysis of the influence of environmental parameters on biological distribution the CANOCO software (ter Braak & Šmilauer, 2002) was used.

2.4.1. Environmental variables

Environmental variables data were square root transformed (for temperature, salinity, ammonium, nitrate, nitrite, phosphate and silicates), or submitted to log (X+1) transformation (for dissolved oxygen and pH), whenever data were moderately skewed in distribution, and followed normalization. A Principal Component Analysis (PCA) of the environmental variables was then performed for ordination, in order to find patterns in multi-dimensional data by reducing the number of dimensions, with minimal loss of information. A resemblance matrix based on Euclidean distance was constructed (Clarke & Green, 1988).

2.4.2. Biological data

In order to test the patchy distribution of meiofauna, an one-way PERMANOVA was previously performed to each set of transects, at each station, in each sampling occasion. This allowed evaluating if the transects at each station differed statistically from one another in terms of meiofauna and nematode composition. Given the high number of tests performed the Dunn-Sidäk correction (Sokal & Rohlf, 1995) was applied.

2.4.2.1. Meiofauna assemblages

Total meiofauna density and density of individual major meiofauna taxa (individuals per 10 cm^2) were calculated, for each station and sampling occasion. Striding to perform multivariate analysis, meiofauna taxa density data were square root transformed in order to scale down densities of highly abundant taxa and therefore increase the importance of the less abundant taxa in the analyses.

To test the hypothesis that the composition of meiofauna changes spatially and seasonally, a two-way PERMANOVA analysis was carried out with the following crossed factor design: station and sampling occasion as fixed factors, with four (It-1, It-2, It-3 and It-4) and three levels (Su-09, Au-09 and Wi-10), respectively. The PERMANOVA test was conducted on Bray–Curtis similarity matrix and the residuals were permutated under a reduced model, with 9999 permutations. The null hypothesis was rejected when the significance level, p, was <0.05 (if the number of permutation was lower than 150, the Monte Carlo permutation p was used). If significant differences were detected, a posteriori pair-wise comparisons, using 9999 permutations under a reduced model were used to examine it. An ordination plot of samples using non-metric

multidimensional scaling (nMDS) was performed on the same matrix. Afterwards, the relative contribution of each taxa to the average dissimilarities between stations and sampling occasions were identified using two-way crossed similarity percentage analysis procedure (SIMPER) (cut-off percentage: 85%).

Statistical differences among stations and seasons were also tested by applying two-way PERMANOVA for the quantitative information of the meiofauna assemblages, namely total density, number of taxa, Margalef index (d), based in the specific richness of a system (Margalef, 1958), and Shannon-Wiener index (H'), that takes into account the proportional abundance of taxa (Shannon & Wiener, 1963); the $\log_2$ was used to compute this index; therefore the results were expressed in bits/individual. Higher values of the indices are an indication of a diverse community. PERMANOVA was used as an alternative to ANOVA since its assumptions were not met, even after data transformation. Likewise, in order to include a index based on ecological strategy, the Nematodes/Copepods Index (Raffaelli & Mason, 1981) was calculated and tested. This index is based on the ratio between the abundances of nematodes and copepods. The values of such ratio can increase or decrease in response to higher or lower organic pollution, which expresses a different response of those groups to the input of organic matter into the system. Values over 100 express high organic pollution (Salas, 2006). For the calculation of the index all the copepods, including nauplii larvae were considered (see Rubal, 2009 for rationale).

2.4.2.2. Nematode assemblages

As the Nematoda is reportedly always the dominant meiofaunal taxon (e.g. (Coull, 1999; Heip et al, 1985), this particular group was studied in more detail.

As for meiofauna data, nematode genera density data were first square root transformed in order to scale down densities of highly abundant genera and therefore increase the importance of the less abundant genera in the analyses. To analyse possible temporal and spatial differences regarding nematode assemblages' composition, the same statistical procedures previously described with regard to meiofauna as a whole were applied (see above).

To investigate the trophic composition of the assemblages, nematode genera were assigned to one of the four functional feeding groups, designated by Wieser (1953), based on buccal cavity morphology (see Annex, Table I): selective (1A) and non-selective (1B) deposit feeders, epigrowth feeders (2A) and omnivores/predators (2B). The Index of Trophic Diversity (Heip et al., 1985) was then calculated as:

$$IDT = \sum \theta^2$$

where θ is the density contribution of each trophic group to total nematode density, ranging from 0.25 (highest trophic diversity, i.e., each of the four trophic guilds account for 25% of the nematode density), to 1.0 (lowest trophic diversity, i.e., one trophic guild accounts for 100% of the nematode density). The Maturity Index (MI) (Bongers, 1990; Bongers et al., 1991) was calculated to analyse changes in the structure and functioning of nematodes assemblages regarding its life strategy. Based on their specific characteristics, all nematode genera

were distributed along a colonizer–persister (c–p) scale (see Annex, Table II). The MI was calculated as the weighted mean of the individual taxon scores:

$$MI = \sum_{i=1}^{n} v(i)f(i)$$

where v(i) is the c–p value of the taxon i (see Annex, Table II) and f(i) is the frequency (per replicate) of that taxon. The index is expressed as a c–p value, ranging from c-p=1 for a colonizer to c–p=5 for a persister, and represents the life-history characteristics associated with r- and K-selection, respectively. Thus, taxa with c–p=1 are r-selected, with short generation times, large population fluctuations, and high fecundity while taxa with c–p=5 are K-selected, producing few offspring and generally appearing later in a given succession (Bongers & Bongers, 1998; Bongers & Ferris, 1999). Low c–p values correspond to taxa that are relatively tolerant to ecological disturbances, unlike taxa with high c–p values, which are sensitive (Neher & Darby, 2009). The MI, in practice, varies from 1, under extremely disturbed conditions, to 3 or 4 under undisturbed conditions.

Univariate measures like total density, genera diversity, trophic composition and several ecological indicators, either based on diversity: Margalef Index (d); Shannon-Wiener diversity (H') or on ecological strategies: ITD and MI, were calculated on the nematode density data of all replicates for each sampling station and occasion. Two-way permutational analyses of variance (PERMANOVA) were applied to test the null hypotheses that no significant spatial (between stations) and temporal (between sampling occasions) differences existed, in the nematode assemblage descriptors All PERMANOVA

tests were conducted on Euclidean-distance similarity matrices and the residuals were permutated under a reduced model, with 9999 permutations. Whenever significant differences were detected, these were examined using a posteriori pair-wise comparisons, using 9999 permutations under a reduced model.

2.4.3. Environmental variables vs. biological data

The relationship between multivariate community structure and environmental variables was explored by means of a redundancy analysis (RDA), since the characteristics of the data, i.e. lengths of gradient < 3, require the use of a linear method (ter Braak & Šmilauer, 2002). As for the environmental variables, only one sample was taken from each station, to perform this statistical procedure, the taxa/genera abundances based on the number of replicates at each station were averaged. Selection of the environmental variables retained in the RDA was performed, by forward selection, using the Monte Carlo permutation test for $p<0.05$ (ter Braak & Šmilauer, 2002). This statistical procedure was also used to determine the significance of the first and all set of canonical axes of the analysis (ter Braak & Šmilauer, 2002).

3. RESULTS

3. RESULTS

3.1. Environmental conditions

In general, the abiotic parameters varied across sampling occasions and throughout the south arm of the Mondego estuary (Table 1). The pH was stable across stations, although slightly lower in summer, especially at stations It-1 and It-2. Dissolved oxygen (DO) presented always lower values in summer and higher in autumn and winter, and in general, it increased towards the upstream stations. Temperature also increased towards the inner stations. However it showed a dramatic change along the south arm in the summer (dry season), with a range of 12 ºC from the downstream to the upstream stretch. Salinity changed dramatically over the seasons, with values around the poli-euhaline class on summer and close to the mesohaline ones on winter (*sensu* Venice System, 1959). Along the south arm, there was a general decrease in this parameter from downstream to upstream stations. Chlorophyll-a (Chl-a) presented to some extent temporal and spatially heterogeneity, with its highest values expressed in autumn. In summer and autumn, It-3 and It-4 were the stations which presented the higher levels of Chl-a, followed by station It-1; nevertheless, in winter, station It-4 presented the lowest value of Chl-a. Most nutrients showed winter peaks, except for the concentration of phosphates, which presented its highest value in autumn. Regarding the spatial distribution, stations It-4 and It-1 showed the highest concentrations of ammonium and silicates. The highest concentration of nitrates was measured at It-3, except in summer, where it peaked at station It-4. For phosphates, the highest values were registered in the upstream sampling sites. In general, the concentration of

nitrites was very low, with the downstream stations exhibiting higher values during summer and winter and the reverse occurring in autumn, with stations It-3 and It-4 showing the highest levels of nitrites. As for the content of organic matter (OM) present in the sediment, the lowest values were always presented at It-3. In general, It-1 and It-4 were the sites with higher OM content. In autumn, the value of OM increased at station It-2, making it as the second richest site in organic matter. As for the sediment grain size, fine sand was the dominant class. It-1 and It-4 were the stations with higher content of silt-clay, whereas stations It-2 and It-3 presented the higher values of mean sand. In autumn, the proportion of coarse sand in the sediments reached its maximum values for all stations, except for It-1.

The PCA ordination of the environmental factors provided a clear distinction of the samples either on space (except for It-1, which in autumn differed from the other seasons) and on sampling occasion (especially the winter) (Fig. 2). Based on data from the environmental parameters, PCA showed that the first two principal components accounted for about 50.4% of the variability of the data: 31.6% was explained by axis 1 and 18.8% by axis 2. Variability along the first axis was mainly explained by an increase in fine and medium sand content in the sediments and a concomitant decrease of gravel and coarse sand, organic matter and silt+clay. Along the second axis the variability was mainly explained by the contrast between sampling stations characterized by higher concentrations of nutrients (except for phosphates), pH and dissolved oxygen *vs.* those with higher proportions of phosphates, salinity and organic matter in the sediments, allowing to separate the samples of winter from the other sampling occasions. The station It-3 was characterized by fine to medium sand,

while It-4 was characterized by coarse sand to gravel sediments, silt-clay and high organic matter content. It-2 and It-1 were more heterogeneous in time. In summer and autumn, It-2 was characterized by higher levels of phosphates and higher salinity, while in winter presented higher concentration of nitrates and temperature. It-1 presented evident seasonal variations: in winter, it was similar to It-4, with higher concentrations of silicates, ammonia, nitrites and silt-clay content in sediment; in autumn, it showed higher salinity and coarse sand sediment; while in summer, presented higher levels of phosphates and salinity.

Table 1 – Environmental variables measured at each sampling station (It-1, It-2, It-3 and It-4), in three occasions (summer, Su-09; autumn, Au-09 and winter, Wi-10) in the Mondego estuary.

Season	Station	pH	DO (mg/L)	T (ºC)	Salinity	$N\text{-}NH_4^+$ (mg/L)	$N\text{-}NO_3^-$ (mg/L)	$N\text{-}NO_2^-$ (mg/L)	PO_4^{3-} (mg/L)	SiO (mg/L)	Chl-a (mg/L)	OM (%)	Gravel (%)	Coarse sand (%)	Mean sand (%)	Fine sand (%)	Silt+clay (%)
Su-09	**It-1**	6.10	5.10	6.00	32.80	0.10	0.16	0.03	0.04	1.34	2.50	5.11	1.35	10.19	10.79	58.04	19.63
	It-2	6.90	4.00	6.30	32.60	0.07	0.23	0.01	0.04	0.63	1.25	2.95	0.52	6.33	12.42	70.84	9.89
	It-3	7.30	6.30	10.60	25.50	0.05	0.94	0.00	0.05	0.54	2.97	2.24	0.12	3.55	15.96	74.92	5.46
	It-4	7.20	7.20	18.30	26.90	0.36	0.44	0.00	0.04	1.05	4.16	4.94	0.41	18.19	11.15	51.90	18.35
Au-09	**It-1**	7.82	11.00	8.10	19.40	0.15	0.22	0.00	0.09	0.83	4.96	6.86	0.10	7.80	12.60	57.90	21.60
	It-2	7.64	9.50	8.60	17.30	0.01	0.05	0.00	0.07	0.99	3.86	6.33	0.70	8.10	21.60	63.40	6.10
	It-3	7.58	11.00	8.80	16.10	0.05	0.41	0.01	0.13	0.60	10.85	2.79	0.40	8.10	12.90	65.70	12.90
	It-4	7.43	10.60	9.20	10.10	0.18	0.12	0.03	0.14	1.69	11.44	5.71	1.60	27.70	13.60	44.20	12.90
Wi-10	**It-1**	7.48	9.80	10.80	8.80	0.14	0.69	0.06	0.00	2.10	2.00	6.66	1.70	8.90	11.70	46.30	31.40
	It-2	7.59	10.10	11.10	6.30	0.09	0.73	0.03	0.00	2.11	3.91	3.27	0.90	4.70	17.80	62.70	13.80
	It-3	7.56	10.90	11.20	4.60	0.15	0.45	0.03	0.01	1.03	9.58	2.13	0.40	1.60	12.80	77.40	7.80
	It-4	7.16	10.60	13.90	5.40	0.26	0.40	0.02	0.01	2.41	1.77	5.16	0.40	7.70	7.20	46.10	38.60

DO, dissolved oxygen; T, temperature; $N\text{-}NH_4^+$, ammonium; $N\text{-}NO_3^-$, nitrate; $N–NO_2^-$, nitrite; $P–PO_4^{3-}$, phosphate; SiO, silicates; Chl-a, chlorophyll-a; OM, sediment organic matter; gravel (>2 mm); coarse sand (0.5–2.0 mm); mean sand (0.25–0.50 mm); fine sand (0.063–0.250 mm); silt + clay (<0.063 mm) (T, DO, Sal, pH, Chl-a and nutrient concentrations were measured in the bottom water).

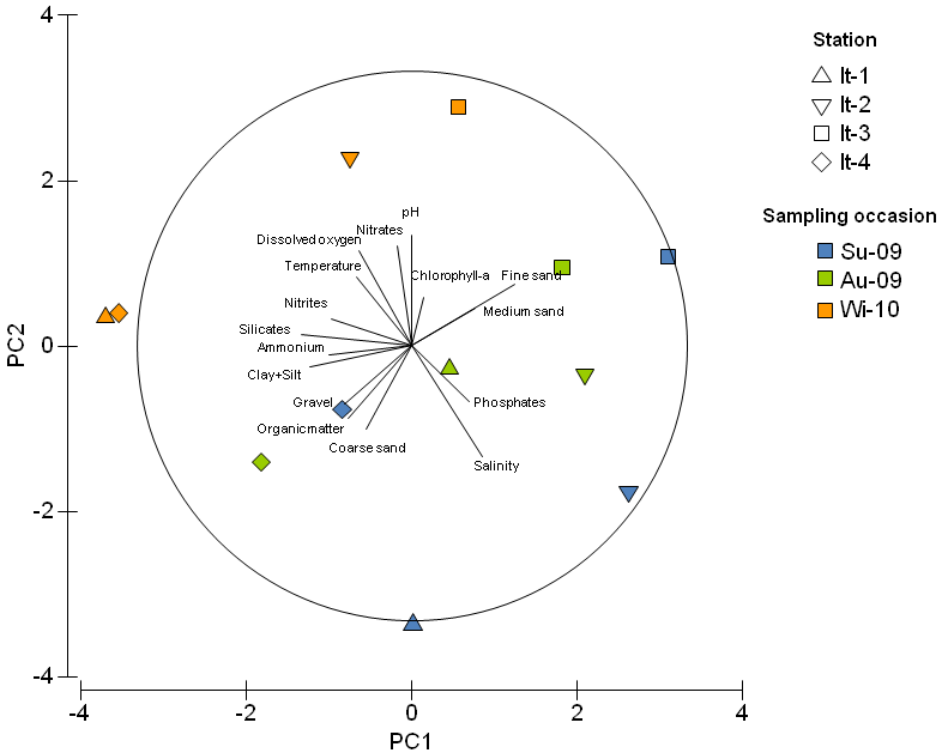

Fig. 2 - Principal Component Analysis (PCA) plot based on the environmental variables measured in each station (It-1, It-2, It-3 and It-4) and sampling occasion (summer, Su-09; autumn, Au-09 and winter, Wi-10).

3.2. Biological data

A prior PERMANOVA analysis revealed that there were no significant differences ($p>0.05$) in respect to meiofauna and nematode composition between the different transects at each sampling station. So, hereafter the biological data will be analysed considering each station as a whole.

3.2.1. Meiofauna assemblages

3.2.1.1. Composition and structure

Thirteen major taxa were identified along the south arm of the Mondego estuary during the sampling period (Table 2). Nematoda was the taxon more abundant (80.6%), followed by Polychaeta (5.2%) and Copepoda (4.9%);

Oligochaeta (2.4%); Gastropoda and Ostracoda (1.6%, each); Turbellaria (1.4%) and Nauplii larvae (1.2%). All other taxa attained less than 1% of the total meiofauna density in the south arm [e.g. Bivalvia (0.6%); Cladocera and Insecta (0.2%, each); Halacaroidea (0.1%) and Crustacea (0.01%)]. Table 2 shows the mean density (number of individuals per $10cm^2$) of meiofauna main taxa, in each station, per sampling occasion. Total meiofauna density (±SD) ranged from 104.96 ± 30.35 ind.10 cm^{-2} (It-1; winter) to 2002.7 ± 1248.85 ind.10 cm^{-2} (It-1; autumn) and the number of taxa present varied from 5 (It-1; winter) to 13 (It-3; autumn).

Table 2 – Mean density ± standard deviation (number of individuals per 10 cm^2) of meiofaunal taxa at each sampling station (It-1, It-2, It-3 and It-4) and sampling occasion (summer, Su-09; autumn, Au-09 and winter, Wi-10).

Sampling occasion	Station	Biv	Cla	Cop	Crust	Gast	Halac	Insec	Nauplii	Nemat	Oligo	Ost	Poly	Turb	Total
Su-09	It-1	2.62 ± 2.3	0.16 ± 0.4	9.5 ± 8.19	0.16 ± 0.4	1.47 ± 1.49		4.91 ± 2.49		505.63 ± 303.48	4.91 ± 2.49		3.11 ± 2.74		532.47 ± 323.98
	It-2	2.29 ± 2.21		6.39 ± 4.68		0.65 ± 0.8		6.55 ± 3.91	0.65 ± 1.19	331.41 ± 103.56	6.55 ± 3.91		4.26 ± 2.12		358.75 ± 122.38
	It-3	15.72 ± 8.39		3.93 ± 1.55		7.07 ± 6.42		1.38 ± 0.88	0.2 ± 0.44	197.86 ± 94.51	1.38 ± 0.88		5.89 ± 2.86		233.43 ± 115.93
	It-4	3.34 ± 2.56		17.49 ± 9.43		0.2 ± 0.44		2.95 ± 3.68	0.79 ± 1.76	579.83 ± 179.03	2.95 ± 3.68		9.63 ± 3.89		617.18 ± 204.47
Au-09	It-1	0.65 ± 1.19	1.15 ± 1.45	131.16 ± 121.53		12.12 ± 12.85	0.65 ± 0.8		19.98 ± 26.54	1747.1 ± 1225.41	6.55 ± 7.21	8.35 ± 6.17	48.14 ± 27.06	26.85 ± 37.39	2002.7 ± 1467.6
	It-2	1.96 ± 1.39	5.4 ± 11.35	45.03 ± 38.18		5.08 ± 3.85	1.64 ± 2.38		46.83 ± 93.4	469.77 ± 200.4	6.39 ± 8.69	16.37 ± 10.34	48.79 ± 23.81	33.57 ± 32.04	680.83 ± 425.83
	It-3	11.79 ± 3.57	3.11 ± 3.48	51.09 ± 26.07	0.49 ± 0.82	79.58 ± 39.36	0.49 ± 0.82	0.16 ± 0.4	14.41 ± 14.83	764.34 ± 416.03	100.37 ± 101.25	38.48 ± 20	131.81 ± 57.02	27.67 ± 17.37	1223.79 ± 701.02
	It-4	3.44 ± 4.47	3.77 ± 2.52	34.22 ± 27.02		0.82 ± 1.15	6.55 ± 6.57		6.39 ± 13.74	875.35 ± 576.96	28.49 ± 36.48	50.6 ± 44.11	109.54 ± 46.97	14.41 ± 13.39	1133.58 ± 773.38
Wi-10	It-1			1.8 ± 3.48		1.31 ± 2.03				90.88 ± 25.11	6.06 ± 4.41		4.91 ± 2.41		104.96 ± 37.44
	It-2	0.16 ± 0.4		11.13 ± 21.57		2.13 ± 2.01				143.6 ± 64.11	2.46 ± 1.84		7.37 ± 4.16		166.85 ± 94.09
	It-3	5.08 ± 4.23		49.78 ± 48.89		4.58 ± 2.12			0.16 ± 0.4	145.89 ± 73.91	5.57 ± 4.29		9.82 ± 11.81		220.88 ± 145.65
	It-4	0.16 ± 0.4		3.44 ± 5.72		0.16 ± 0.4				190.92 ± 59.16	1.64 ± 1.48	0.33 ± 0.51	3.93 ± 1.24		200.58 ± 68.91

Biv, Bivalvia; Cla, Cladocera; Cop, Copepoda; Crust, Crustacea; Halac, Halacaroidea; Insec, Insecta; Nemat, Nematoda; Oligo, Oligochaeta; Ost, Ostracoda; Poly, Polychaeta; Turb, Turbellaria.

The nMDS plot clearly reflected the temporal distribution of meiofauna along the south arm of Mondego estuary, according to the density of the different taxa; spatially this separation was less evident (Fig. 3). Multivariate PERMANOVA analysis of meiofauna assemblage composition data supported these distribution patterns. Meiofauna composition differed significantly among sampling occasions (Pseudo-F=57.942, df=2, P(perm)=0.001), and also between stations (Pseudo-F=5.4644, df=3, P(perm)=0.001), although to a lesser extent. There was also a significant interaction between both factors (Pseudo-F=2.7273, df=6, P(perm)=0.001), indicating that temporal trends were not consistent across stations.

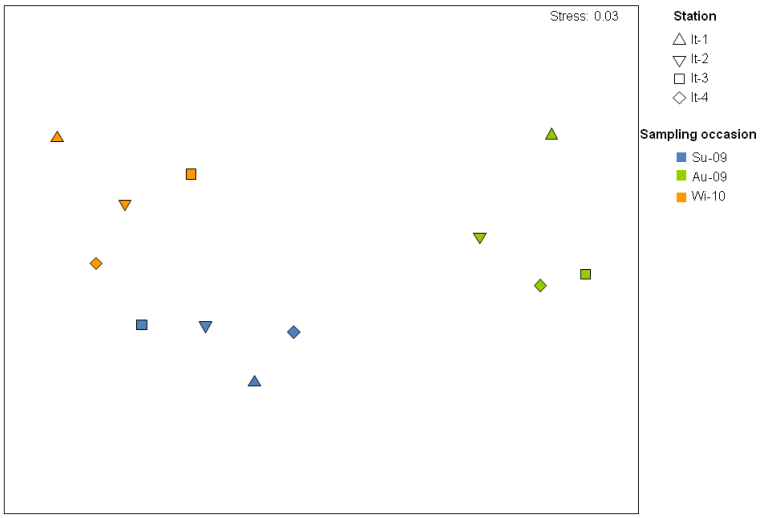

Fig. 3 - Non-metric multidimensional scaling (nMDS) ordination plot, based on the meiofauna density, of each sampling station (It-1, It-2, It-3 and It-4) and each sampling occasion (summer, Su-09; autumn, Au-09 and winter, Wi-10).

Two-way SIMPER analysis showed that 10 taxa groups explained 80% of the similarities within and between each station and sampling occasion group, and showed a low mean dissimilarity between the four stations and the three sampling occasions, as the maximum dissimilarities obtained were 33.05%

(between It-1 and It-3) and 55.49 (between autumn and winter), respectively (Table 3A and 3B). The taxa that contributed the most to the similarity for both sampling occasions and stations were Nematoda, Polychaeta, Copepoda and Oligochaeta.

Table 3 – Major taxa groups determined by SIMPER analysis as contributing the most to the similarity/dissimilarity of meiofauna communities within (A) stations and (B) sampling occasions. Shaded boxes: percent similarity (bold) and the taxa that contributed to the similarity in each group. Non-shaded box, percent dissimilarity (bold) and the species that contributed to the total dissimilarity (cut-off percentage: 85%).

(A)	It-1		It-2		It-3		It-4	
It-1	**76.2**							
	Nematoda	66.77%						
	Polychaeta	9.39%						
	Oligochaeta	8.32%						
	Copepoda	6.96%						
It-2	**24.97**		**77.16**					
	Nematoda	40.53%	Nematoda	60.96%				
	Copepoda	16.77%	Polychaeta	11.57%				
	Polychaeta	7.85%	Copepoda	7.08%				
	Gastropoda	7.83%	Oligochaeta	6.46%				
	Oligochaeta	7.49%						
	Nauplii	5.41%						
It-3	**33.05**		**29.29**		**76.91**			
	Nematoda	32.52%	Nematoda	25.75%	Nematoda	44.88%		
	Copepoda	18.01%	Copepoda	16.33%	Copepoda	11.72%		
	Gastropoda	10.81%	Gastropoda	12.5%	Polychaeta	11.52%		
	Bivalvia	10.43%	Oligochaeta	11.28%	Gastropoda	9.55%		
	Oligochaeta	9.1%	Bivalvia	11.06%	Bivalvia	7.76%		
	Polychaeta	7.93%	Polychaeta	8.65%				
It-4	**26.71**		**24.58**		**31.06**		**79.73**	
	Nematoda	41.01%	Nematoda	33.54%	Nematoda	30.31%	Nematoda	66.98%
	Copepoda	13.54%	Copepoda	14.83%	Copepoda	17.47%	Polychaeta	13.27%
	Oligochaeta	10.32%	Polychaeta	10.06%	Gastropoda	14.66%	Copepoda	6.78%
	Polychaeta	8.55%	Oligochaeta	9.47%	Oligochaeta	9.65%		
	Gastropoda	6.34%	Gastropoda	7.06%	Bivalvia	9.24%		
	Ostracoda	5.15%	Nauplii	5.92%	Polychaeta	6.17%		
	Nauplii	3.46%	Ostracoda	4.72%				

(B)	Su-09		Au-09		Wi-10	
Su-09	**81.37**					
	Nematoda	66.43%				
	Copepoda	7.83%				
	Polychaeta	6.77%				
	Insecta	5.56%				
Au-09	**44.58**		**73.83**			
	Nematoda	24.23%	Nematoda	66.43%		
	Polychaeta	14.1%	Copepoda	7.83%		
	Ostracoda	10.29%	Polychaeta	6.77%		
	Copepoda	10.28%	Insecta	5.56%		
	Turbellaria	9.65%				
	Oligochaeta	7.03%				
	Nauplii	6.68%				
	Gastropoda	5.79%				
Wi-10	**35.16**		**55.49**		**77.82**	
	Nematoda	46.86%	Nematoda	34.1%	Nematoda	67.84%
	Copepoda	15.34%	Polychaeta	12.79%	Polychaeta	12.32%
	Insecta	10.29%	Copepoda	10.72%	Oligochaeta	8.55%
	Bivalvia	8.53%	Ostracoda	9.4%		
	Oligochaeta	5.91%	Turbellaria	8.63%		
			Oligochaeta	6.03%		
			Nauplii	5.89%		

3.2.1.2. Indices estimation

The total density of meiofauna was clearly higher in autumn and lower in winter (Fig. 4A). PERMANOVA confirmed these temporal variations, but also spatial ones (Table 4). It was also detected a significant interaction between both factors (Table 4, see Appendix, Table I). Regarding the number of taxa, the higher richness was also observed in autumn and the lower values in winter. In this case, station It-3 presented higher values relative to all other stations (Fig. 4B). PERMANOVA revealed differences between all pairs of sampling occasions (Table 4). The significant differences ($p<0.05$) between stations was accounted for the higher number of taxa presented at It-3 relative to all other stations (see Appendix, Table I). The Margalef and the Shannon-Wiener indices showed the same tendency as the taxa richness: more diversity in autumn, and lower in winter, with It-3 seemingly the higher diverse station across seasons (Fig. 4C and 4D, respectively). PERMANOVA confirmed statistical differences among stations and across sampling stations (Table 4). Also, for both indices a significant interaction between both factors was detected (Table 4, see Appendix, Table I). As for Nematode/Copepod Index, there were only differences between sampling occasions (Table 4), specifically because of the relatively higher ratio that occurred in summer (Fig. 4E, see Appendix, Table I).

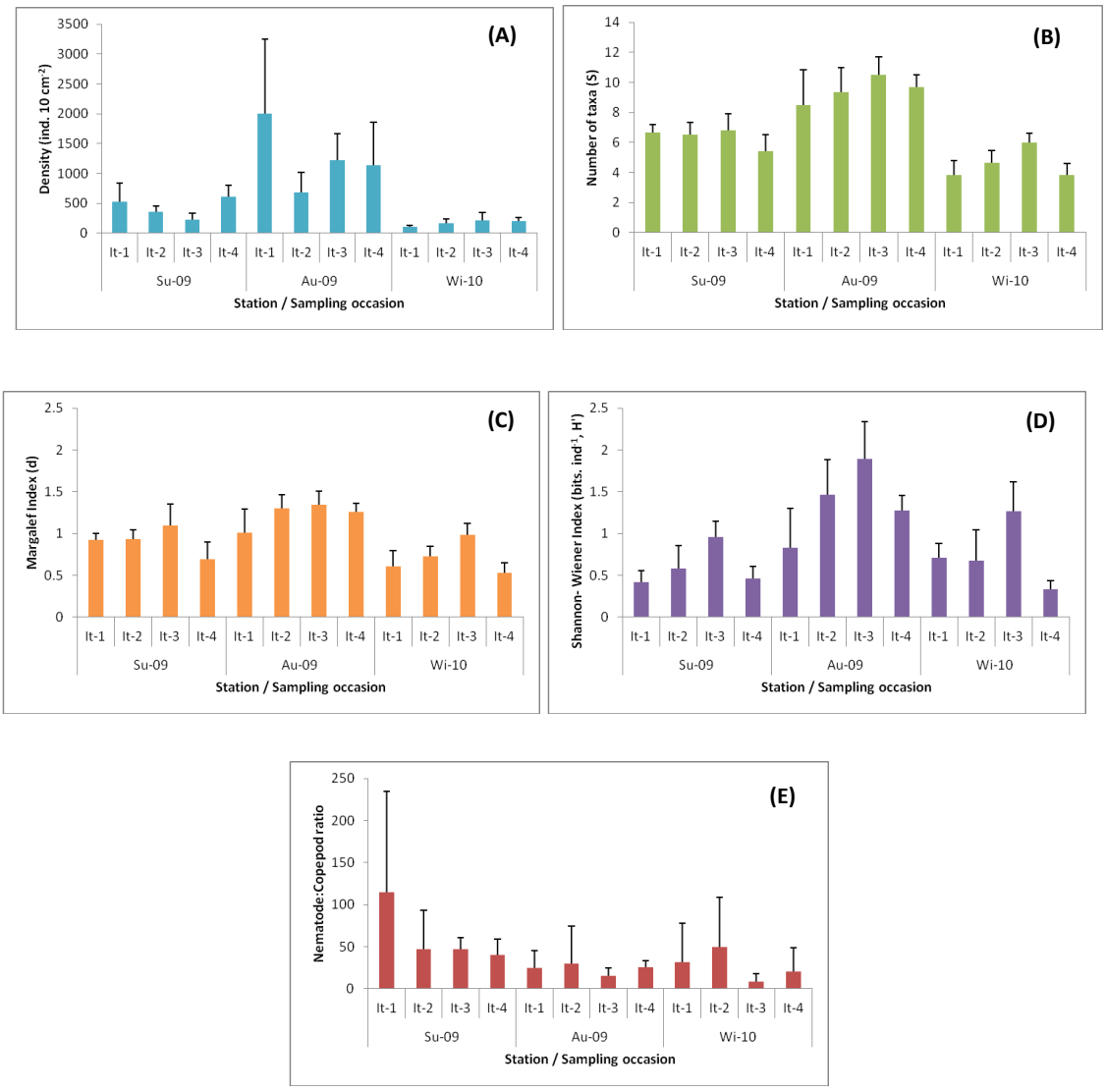

Fig. 4 - Meiofauna (A) Mean density ± SD (ind. 10 cm^{-2}); (B) Mean number of taxa ± SD; (C) Mean Margalef index ± SD; (D) Mean Shannon-Wiener index (bits. ind^{-1}) ± SD; (E) Mean nematode to copepod ratio ± SD assessed at each sampling station, in each sampling occasion.

Table 4 - Details of the univariate two-factor PERMANOVA test for all meiofauna descriptors analysed. Bold values stand for the significant differences ($p < 0.05$).

	Source of variation	Degrees of freedom	Pseudo-F	P(perm)
Total density	Sampling occasion	2	34.797	**0.001**
	Station	3	3.2438	**0.020**
	Sampling occasion x Station	6	2.8749	**0.012**
Number of taxa	Sampling occasion	2	108.5	**0.001**
	Station	3	5.8188	**0.003**
	Sampling occasion x Station	6	1.5545	0.166
Margalef Index	Sampling occasion	2	56.7	**0.001**
	Station	3	12.656	**0.001**
	Sampling occasion x Station	6	2.6313	**0.027**
Shannon-Wiener Index	Sampling occasion	2	41.757	**0.001**
	Station	3	20.445	**0.001**
	Sampling occasion x Station	6	2.4151	**0.031**
Nematode/Copepod Ratio	Sampling occasion	2	4.6277	**0.010**
	Station	3	1.5791	0.202
	Sampling occasion x Station	6	1.1143	0.387

3.2.2. Nematode assemblages

3.2.2.1. Composition and Structure

Forty-six genera of nematodes belonging to seventeen families were identified (Table 5; Annex, Table II). The more abundant families were Comesomatidae, Chromadoridae, Xyalidae, Linhomoeidae, Sphaerolaimidae, Oncholaimidae, Desmodoridae and Anoplostomatidae. The genera *Sabatieria* (24.2%), *Daptonema* (15.2%), *Sphaerolaimus* (10.3%), *Ptycholaimellus* (9.3%), *Viscosia* (5.9%), *Dichromadora* (4.4%), *Paralinhomoeus* (4.2%), *Terschellingia* (4.1%), *Metachromadora* (3.1%), *Anoplostoma* (2.6%), *Cromadora* (2.0%), *Desmolaimus* (1.6%), *Microlaimus* (1.5%) and *Axonolaimus* (1.3%) together represented 90% of the total nematode density. Table 5 shows the mean density of nematodes, in each station, per sampling occasion. Total nematode density (± SD) varied from 90.88 ± 25.11 ind. 10 cm^{-2}, during winter, at It-1 to 1753.45 ± 1205.32 ind. 10 cm^{-2} during autumn also at station It-1, with It-4 during summer presenting the lowest richness (15 genera) and It-3, during autumn the highest one (37 genera). Among the more abundant genera, eight genera presented ubiquity; i.e. appeared in all sampling occasions, at all the stations, namely *Sabatieria*, *Daptonema*, *Sphaerolaimus*, *Viscosia*, *Dichromadora*, *Paralinhomoeus*, *Terschellingia* and *Anoplostoma*. In contrast, eight other genera only appeared in one sampling occasion in one station: in summer, *Aponema* appeared at It-2, *Bathylaimus* at It-3 and *Spilophorella* at It-4; in autumn, *Oncholaimus* and *Cyatholaimus* only apperared at It-1, *Comesoma* at It-2 and *Chromadorella* at It-3. In winter, *Hypodontalaimus* appeared only at It-4.

Table 5 - Mean density ± standard deviation (number of individuals per 10 cm^2) of nematode genera in each sampling station (It-1, It-2, It-3 and It-4) and sampling occasion (summer, Su-09; autumn, Au-09 and winter, Wi-10). The five most abundant genera in each area are bolded.

Genera (code)		Mean density	Su-09				Au-09				Wi-10			
			It-1	It-2	It-3	It-4	It-1	It-2	It-3	It-4	It-1	It-2	It-3	It-4
Sabatieria	(1)	**120.53 ± 157.39**	**167.85 ± 120.24**	**44.83 ± 7.97**	**93.3 ± 56.71**	**280.45 ± 137.81**	**402.31 ± 288.52**	**37.8 ± 30.79**	**177.23 ± 99.55**	**203.4 ± 100.21**	**17.17 ± 12.88**	5.56 ± 2.76	**10.47 ± 9.51**	**28.09 ± 14.85**
Daptonema	(2)	**77.44 ± 73.49**	**78.73 ± 73.73**	**83.09 ± 51.66**	**13.46 ± 5.14**	**87.76 ± 49.25**	**141.82 ± 78.93**	**86.04 ± 69.79**	**69.07 ± 27.67**	**171.73 ± 156.29**	**33.88 ± 12.84**	**33.93 ± 26.15**	**50.06 ± 31.25**	**70.8 ± 28.03**
Sphaerolaimus	(3)	**52.18 ± 69.69**	**40.7 ± 12.36**	**24.18 ± 16.18**	**18.2 ± 5.49**	**86.12 ± 35.34**	**169.58 ± 118.05**	**40.74 ± 31.16**	**48.95 ± 30.23**	**139.65 ± 114.07**	**7.28 ± 2.2**	**14.89 ± 8.7**	**13.13 ± 13.49**	**22.76 ± 15.64**
Ptycholaimellus	(4)	**47.76 ± 108.93**	5.73 ± 8.23	20.27 ± 14.67	4.69 ± 5.37	**42.79 ± 36.99**	**271.53 ± 270.42**	**47.94 ± 33.13**	21.34 ± 12.74	**97.43 ± 101.7**		**14.95 ± 19.86**	5.82 ± 7.86	**32.63 ± 44.37**
Viscosia	(5)	**30.42 ± 40.1**	12.31 ± 12.59	8.37 ± 10.49	**16.72 ± 5.84**	16.41 ± 9.95	52.53 ± 85.59	**49.86 ± 53.21**	**92.89 ± 32.03**	54.49 ± 26.59	**5.09 ± 1.36**	**14.28 ± 5.26**	**28.38 ± 20.53**	9.07 ± 7.79
Dichromadora	(6)	22.65 ± 36.04	23.79 ± 21.8	13.88 ± 13.58	5.76 ± 4.94	12.45 ± 7.35	**96.61 ± 88.58**	30.87 ± 17.23	20.63 ± 19.84	11.55 ± 6.2	**17.21 ± 10.05**	**28.13 ± 15.3**	5.37 ± 4.21	0.98 ± 1.24
Paralinhomoeus	(7)	21.64 ± 37.65	**26.85 ± 29.84**	**23.13 ± 23.33**	4 ± 3.8	**20.67 ± 12.61**	65.68 ± 76.24	22.73 ± 20.79	**47.96 ± 67.02**	36.68 ± 40.46	2.77 ± 1.94	2.19 ± 2.12	0.99 ± 1.09	2.94 ± 2.98
Terschellingia	(8)	21.1 ± 44.12	**87.92 ± 82.95**	13.44 ± 13.07	**21.88 ± 20.65**	6.49 ± 8.65	76.3 ± 79.52	2.45 ± 2.77	16.66 ± 33.93	20.99 ± 16.29	1.1 ± 1.33	0.38 ± 0.92	0.35 ± 0.86	2.95 ± 3.11
Metachromadora	(9)	15.8 ± 35.63	0.56 ± 1.36			4.11 ± 3.76	66.13 ± 71.18	16.19 ± 11.61	9.6 ± 7.96	**73.99 ± 57.08**	2.02 ± 2.35	0.79 ± 1.93	1.12 ± 1.82	**10.51 ± 7.94**
Anoplostoma	(10)	13.41 ± 31.2	8.68 ± 16.28	6.78 ± 2.62	8.52 ± 3.9	10.24 ± 14.37	67.09 ± 91.39	6.53 ± 7.43	21.75 ± 110.36	9.86 ± 7.48	0.19 ± 0.48	2.43 ± 3.44	**13.87 ± 12.93**	3.62 ± 6
Chromadora	(11)	10.29 ± 27.18	1.56 ± 3.83	9.41 ± 21.63	0.2 ± 0.44		62 ± 68.47	18.86 ± 15.48	13.18 ± 8.73	14.32 ± 25.68	0.49 ± 0.77	1.27 ± 2.52	0.18 ± 0.43	0.58 ± 0.99
Desmolaimus	(12)	8.31 ± 46.18		0.79 ± 1.93	0.39 ± 0.88		74.46 ± 151.4	3.06 ± 4.94	15.93 ± 21.51	1.97 ± 3.05			0.46 ± 0.74	
Microlaimus	(13)	8 ± 28.99		1.26 ± 3.09			67.01 ± 80.55	9.37 ± 6.52	14.07 ± 14.56				1.4 ± 1.74	0.18 ± 0.44
Axonolaimus	(14)	6.85 ± 15.07	2.67 ± 5.08	3.89 ± 3.88		1.4 ± 3.14	24.98 ± 32.48	12.57 ± 12.33	12.88 ± 27.82	11.97 ± 16.5	0.19 ± 0.48	7.89 ± 8.29	1.2 ± 1.44	0.51 ± 1.24
Paracomesoma	(15)	6.22 ± 13.99	22.44 ± 14.33	**41.02 ± 17.66**	0.89 ± 1.5		0.79 ± 1.95	5.24 ± 6.45	2.29 ± 3.69					
Linhomoeus	(16)	5.8 ± 10.08	15 ± 15.26	10.58 ± 8.37	0.88 ± 1.2	4.13 ± 6.19	16.33 ± 21.56	9.23 ± 9.58	7.59 ± 7.08		0.86 ± 1.04	2.17 ± 1.96	0.98 ± 1.55	0.76 ± 1.18
Leptolaimus	(17)	4.97 ± 16.31		0.83 ± 2.04			44.34 ± 36.05	3.11 ± 6.88		9.47 ± 13.59		0.19 ± 0.46		
Nemanema	(18)	4.27 ± 12.45		0.8 ± 1.95	2 ± 2.9	2.7 ± 6.04		3.63 ± 5.62	34.97 ± 28.06	1.02 ± 2.5		2.38 ± 2.66	2.39 ± 2.95	0.67 ± 1.2
Halalaimus	(19)	4.07 ± 12.37		2.54 ± 3.93	0.86 ± 0.95		3.02 ± 7.39	14.9 ± 19.13	19.7 ± 31.74	5.97 ± 10.15	0.21 ± 0.51			0.4 ± 0.98
Metalinhomoeus	(20)	3.3 ± 10.42	8.65 ± 17.33	1.83 ± 2.15	8.91 ± 8.2		12.06 ± 29.54	1.9 ± 4.65	2.83 ± 4.43	1.02 ± 2.5	0.88 ± 1.05	0.52 ± 1.29	0.7 ± 1.73	0.73 ± 1.25
Calyptronema	(21)	3.24 ± 9.14	1.67 ± 4.08	14.09 ± 17.81		1.4 ± 3.14	1.4 ± 3.44	4.5 ± 4.12	9.24 ± 22.63		0.65 ± 1.11	3.91 ± 4.48		1.12 ± 1.74
Eleutherolaimus	(22)	3.2 ± 12.38		3.12 ± 3.69	0.81 ± 1.1			9.23 ± 9.07	20.83 ± 38.34	2.76 ± 6.75	0.19 ± 0.48	0.48 ± 0.75		
Odontophora	(23)	2.45 ± 7.73			0.86 ± 0.95		1.42 ± 3.47	2.85 ± 6.98	19.75 ± 18.59	1.97 ± 3.05		1.01 ± 1.58	0.92 ± 1.48	

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Table 5 continued

Genera (code)		Mean density	Su-09				Au-09				Wi-10			
			It-1	It-2	It-3	It-4	It-1	It-2	It-3	It-4	It-1	It-2	It-3	It-4
Calyptronema	(21)	3.24 ± 9.14	1.67 ± 4.08	14.09 ± 17.81		1.4 ± 3.14	1.4 ± 3.44	4.5 ± 4.12	9.24 ± 22.63		0.65 ± 1.11	3.91 ± 4.48		1.12 ± 1.74
Eleutherolaimus	(22)	3.2 ± 12.38		3.12 ± 3.69	0.81 ± 1.1			9.23 ± 9.07	20.83 ± 38.34	2.76 ± 6.75	0.19 ± 0.48	0.48 ± 0.75		
Odontophora	(23)	2.45 ± 7.73			0.86 ± 0.95		1.42 ± 3.47	2.85 ± 6.98	19.75 ± 18.59	1.97 ± 3.05		1.01 ± 1.58	0.92 ± 1.48	
Molgolaimus	(27)	1.24 ± 5.95					4.48 ± 10.98		6.93 ± 16.97	0.94 ± 2.31			2.06 ± 2.85	
Antomicron	(28)	1.09 ± 2.82			0.39 ± 0.54		2.82 ± 4.37	2.41 ± 4.37	3.72 ± 6.01	1.83 ± 2.84	0.21 ± 0.51		1.36 ± 1.97	
Paracyatholaimus	(29)	1.03 ± 3.36	0.52 ± 1.28		0.61 ± 0.92		3.02 ± 7.39	3.64 ± 6.56	2.31 ± 5.66		0.25 ± 0.62	0.93 ± 1.12	0.28 ± 0.7	0.51 ± 0.8
Prochromadorella	(30)	0.95 ± 4.74		0.39 ± 0.97	0.39 ± 0.54				9.29 ± 14.47	1.02 ± 2.5				
Paracanthonchus	(31)	0.84 ± 3.62					6.76 ± 10.65	3.05 ± 3.37						
Aegialoalaimus	(32)	0.49 ± 2.08						1.9 ± 4.65	1.45 ± 3.54	1.68 ± 4.11			0.64 ± 0.99	
Camacolaimus	(33)	0.46 ± 1.92					1.42 ± 3.47		2.98 ± 4.63	1.02 ± 2.5				
Chromadorina	(34)	0.44 ± 1.78			0.2 ± 0.44		1.42 ± 3.47		2.32 ± 3.76				1.25 ± 3.05	
Araeolaimus	(35)	0.39 ± 2.46						1.41 ± 3.46	3.14 ± 7.69					
Oncholaimus	(36)	0.38 ± 3.21					4.48 ± 10.98							
Chromadorella	(37)	0.26 ± 1.5							2.98 ± 4.63					
Thalassoalaimus	(38)	0.24 ± 2.03			0.59 ± 1.32				2.31 ± 5.66					
Cyatholaimus	(39)	0.24 ± 1.69					2.83 ± 6.93							
Spilophorella	(40)	0.2 ± 1.21				2.7 ± 6.04								
Cyartonema	(41)	0.19 ± 1.62						0.95 ± 2.33	1.41 ± 3.46					
Theristus	(42)	0.18 ± 0.91							0.88 ± 2.15	0.89 ± 2.18				0.29 ± 0.71
Aponema	(43)	0.07 ± 0.6		0.83 ± 2.04										
Hypodontolaimus	(44)	0.07 ± 0.58												0.8 ± 1.97
Comesoma	(45)	0.05 ± 0.44						0.61 ± 1.49						
Bathylaimus	(46)	0.01 ± 0.12			0.2 ± 0.44									

The nMDS ordination plot clearly revealed temporal differences in nematodes density, but spatially this separation was less evident (Fig. 6). PERMANOVA analysis of nematode assemblage composition data detected both temporal (Pseudo-F=19.581, df=2, P(perm)=0.001) and spatial (Pseudo-F=7.1683, df=3, P(perm)=0.001) significant differences. There was also a significant interaction between both factors (Pseudo-F=2.4968, df=6, P(perm)=0.001), indicating that temporal trends were not consistent across stations.

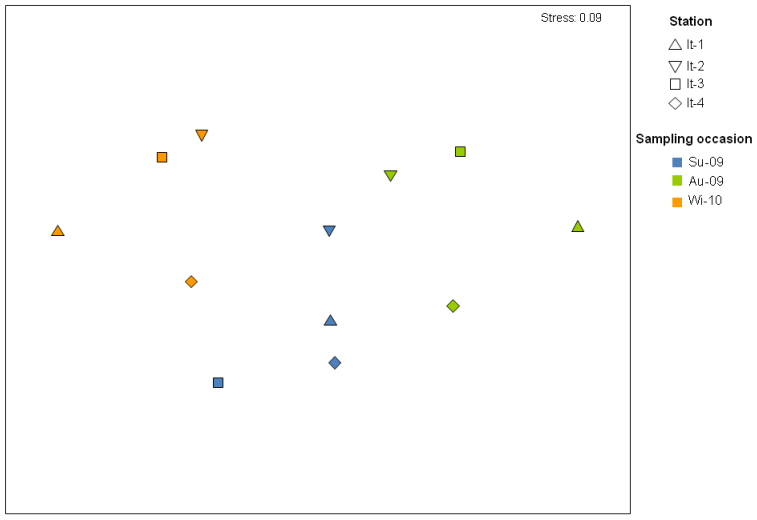

Fig. 6 - Non-metric multidimensional scaling (nMDS) ordination plot based on the nematode density at each sampling station (It-1, It-2, It-3 and It-4) and in each sampling occasion (summer, Su-09; autumn, Au-09 and winter, Wi-10).

Two-way SIMPER analysis showed how the nematodes genera contributed to similarity values of the assemblages among stations and sampling occasions (Table 6A and 6B). Among sampling occasions, maximum dissimilarities were obtained between winter and autumn (57%) and summer (50%). All stations showed around 40% of dissimilarity values. The genera that contributed most to the similarity within both sampling occasions and stations were *Sabatieria*, *Daptonema* and *Sphaerolaimus*.

Table 6 – Nematode genera determined by SIMPER analysis as contributing the most to the similarity/dissimilarity of nematode communities within (A) sampling occasions and (B) stations. Shaded boxes: percent similarity (bold) and the genera that contributed to the similarity in each group. Non-shaded box: percent dissimilarity (bold) and the genera that contributed to the total dissimilarity (cut-off percentage: 85%).

(A)	Su-09		Au-09		Wi-10	
Su-09	**66.96**					
	Sabatieria	23.91%				
	Daptonema	14.46%				
	Sphaerolaimus	13.12%				
	Paracomesoma	6.67%				
	Terschellingia	6.64%				
	Paralinhomoeus	6.42%				
	Viscosia	6.01%				
	Dichromadora	5.44%				
	Ptycholaimellus	5.4%				
Au-09	**47.56**		**60.23**			
	Ptycholaimellus	6.83%	*Sabatieria*	15.44%		
	Sabatieria	6.37%	*Daptonema*	12.4%		
	Daptonema	6.32%	*Sphaerolaimus*	11.04%		
	Metachromadora	6.02%	*Ptycholaimellus*	9.05%		
	Viscosia	5.58%	*Viscosia*	8.77%		
	Chromadora	4.89%	*Dichromadora*	6.24%		
	Terschellingia	4.86%	*Metachromadora*	5.39%		
	Sphaerolaimus	4.63%	*Paralinhomoeus*	5.1%		
	Paralinhomoeus	4.37%	*Anoplostoma*	3.92%		
	Dichromadora	3.82%	*Chromadora*	3.79%		
	Paracomesoma	3.54%	*Terschellingia*	2.58%		
	Axonolaimus	3.43%	*Microlaimus*	2.2%		
	Microlaimus	3.39%				
	Anoplostoma	3.36%				
	Desmolaimus	2.9%				
	Linhomoeus	2.69%				
	Leptolaimus	2.56%				
	Halalaimus	2.48%				
	Nemanema	2.18%				
	Metalinhomoeus	2%				
	Eleutherolaimus	1.74%				
	Odontophora	1.66%				
Wi-10	**49.47**		**57.23**		**63.95**	
	Sabatieria	16.64%	*Sabatieria*	10.89%	*Daptonema*	24.93%
	Terschellingia	9.1%	*Ptycholaimellus*	7.39%	*Sphaerolaimus*	13.52%
	Daptonema	7.91%	*Sphaerolaimus*	6.54%	*Sabatieria*	13.48%
	Paracomesoma	6.77%	*Daptonema*	5.55%	*Viscosia*	11.48%
	Sphaerolaimus	6.75%	*Paralinhomoeus*	5.42%	*Dichromadora*	11.32%
	Paralinhomoeus	5.82%	*Viscosia*	5.16%	*Ptycholaimellus*	6.34%
	Dichromadora	4.87%	*Metachromadora*	5.15%	*Anoplostoma*	3.72%
	Ptycholaimellus	4.67%	*Chromadora*	4.57%	*Metachromadora*	3.21%
	Viscosia	4.29%	*Terschellingia*	3.93%		
	Linhomoeus	3.89%	*Dichromadora*	3.62%		
	Anoplostoma	3.64%	*Axonolaimus*	3.61%		
	Metalinhomoeus	3.28%	*Anoplostoma*	3.52%		
	Axonolaimus	2.55%	*Microlaimus*	3.15%		
	Calyptronema	2.32%	*Desmolaimus*	2.67%		
	Metachromadora	2.09%	*Halalaimus*	2.58%		
	Chromadora	1.95%	*Leptolaimus*	2.39%		
			Nemanema	2.19%		
			Linhomoeus	2.06%		
			Odontophora	1.85%		
			Eleutherolaimus	1.74%		
			Oxystomina	1.7%		

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Table 6 continued

(B)	It-1		It-2		It-3		It-4	
It-1	**64.04**							
	Sabatieria	19.99%						
	Daptonema	18.93%						
	Sphaerolaimus	13.7%						
	Dichromadora	10.4%						
	Viscosia	6.18%						
	Paralinhomoeus	6.1%						
	Terschellingia	5.78%						
	Ptycholaimellus	3.61%						
	Paracomesoma	2.92%						
It-2	**44.69**		**62.3**					
	Sabatieria	9.8%	*Daptonema*	15.29%				
	Ptycholaimellus	8.42%	*Sabatieria*	10.97%				
	Daptonema	6.6%	*Dichromadora*	10.81%				
	Terschellingia	6.52%	*Sphaerolaimus*	9.97%				
	Dichromadora	5.39%	*Ptycholaimellus*	8.97%				
	Sphaerolaimus	4.77%	*Viscosia*	8.5%				
	Viscosia	4.71%	*Paracomesoma*	5.19%				
	Axonolaimus	4.47%	*Paralinhomoeus*	4.17%				
	Paralinhomoeus	4.39%	*Linhomoeus*	3.74%				
	Anoplostoma	4.09%	*Axonolaimus*	3.19%				
	Calyptronema	3.86%	*Anoplostoma*	3.17%				
	Linhomoeus	3.63%	*Calyptronema*	2.93%				
	Chromadora	3.32%						
	Metachromadora	2.85%						
	Oncholaimellus	2.74%						
	Metalinhomoeus	2.57%						
	Eleutherolaimus	2.18%						
	Nemanema	2.15%						
	Leptolaimus	2.09%						
	Microlaimus	1.95%						
It-3	**47.68**		**46.31**		**61.15**			
	Viscosia	7.21%	*Daptonema*	7.69%	*Sabatieria*	16.62%		
	Sabatieria	7.04%	*Sabatieria*	5.97%	*Daptonema*	15.44%		
	Daptonema	6.99%	*Viscosia*	5.73%	*Viscosia*	13.1%		
	Anoplostoma	6.56%	*Dichromadora*	5.48%	*Sphaerolaimus*	11.13%		
	Ptycholaimellus	6.14%	*Ptycholaimellus*	4.99%	*Anoplostoma*	9.42%		
	Terschellingia	5.9%	*Paralinhomoeus*	4.43%	*Dichromadora*	5.74%		
	Dichromadora	5.71%	*Calyptronema*	4.3%	*Ptycholaimellus*	4.5%		
	Paralinhomoeus	4.25%	*Axonolaimus*	4.25%	*Paralinhomoeus*	3.24%		
	Sphaerolaimus	4.23%	*Anoplostoma*	4.19%	*Nemanema*	2.81%		
	Nemanema	3.39%	*Paracomesoma*	4.14%	*Terschellingia*	2.76%		
	Linhomoeus	3.33%	*Nemanema*	3.73%	*Odontophora*	2.16%		
	Metalinhomoeus	3.13%	*Sphaerolaimus*	3.63%				
	Axonolaimus	2.92%	*Linhomoeus*	3.52%				
	Metachromadora	2.91%	*Terschellingia*	2.94%				
	Paracomesoma	2.59%	*Oncholaimellus*	2.83%				
	Microlaimus	2.37%	*Odontophora*	2.7%				
	Chromadora	2.09%	*Chromadora*	2.54%				
	Odontophora	2.08%	*Oxystomina*	2.41%				
	Desmolaimus	2.04%	*Eleutherolaimus*	2.3%				
	Chromadorita	1.81%	*Metalinhomoeus*	2.17%				
	Leptolaimus	1.66%	*Halalaimus*	1.89%				
	Antomicron	1.6%	*Chromadorita*	1.89%				
			Microlaimus	1.84%				
It-4	**42.1**		**46.37**		**45.58**		**66.69**	
	Ptycholaimellus	11.97%	*Sabatieria*	11.77%	*Sabatieria*	8.67%	*Sabatieria*	21.96%
	Sabatieria	8.56%	*Daptonema*	7.94%	*Daptonema*	8.33%	*Daptonema*	20.13%
	Daptonema	8.29%	*Dichromadora*	6.52%	*Ptycholaimellus*	7.92%	*Sphaerolaimus*	15.38%
	Dichromadora	7.8%	*Sphaerolaimus*	6.47%	*Sphaerolaimus*	6.88%	*Ptycholaimellus*	10.71%
	Terschellingia	7.06%	*Ptycholaimellus*	6.26%	*Metachromadora*	6.07%	*Viscosia*	8.3%
	Sphaerolaimus	6.06%	*Metachromadora*	5.7%	*Viscosia*	5.22%	*Metachromadora*	7.08%
	Metachromadora	5.35%	*Viscosia*	4.04%	*Anoplostoma*	4.95%	*Paralinhomoeus*	5.56%
	Viscosia	4.87%	*Axonolaimus*	4.01%	*Terschellingia*	4.63%		
	Anoplostoma	4.63%	*Terschellingia*	4%	*Paralinhomoeus*	4.3%		
	Paralinhomoeus	4.06%	*Paracomesoma*	3.99%	*Nemanema*	3.91%		
	Linhomoeus	3.35%	*Paralinhomoeus*	3.83%	*Dichromadora*	3.49%		
	Chromadora	2.94%	*Calyptronema*	3.63%	*Metalinhomoeus*	2.64%		
	Paracomesoma	2.75%	*Linhomoeus*	3.4%	*Linhomoeus*	2.48%		
	Axonolaimus	2.64%	*Anoplostoma*	3.27%	*Axonolaimus*	2.42%		
	Metalinhomoeus	2.43%	*Chromadora*	2.85%	*Odontophora*	2.26%		
	Microlaimus	2.14%	*Oncholaimellus*	2.84%	*Microlaimus*	2.15%		
	Calyptronema	1.77%	*Nemanema*	2.19%	*Chromadorita*	1.89%		
			Eleutherolaimus	2.12%	*Halalaimus*	1.86%		
			Halalaimus	1.95%	*Chromadora*	1.81%		
					Desmolaimus	1.65%		
					Antomicron	1.59%		

3.2.2.2. Trophic Composition

In the south arm subsystem of Mondego estuary, non-selective deposit feeders (1B: 52.89 ± 9.37%, 12 genera) were the most abundant feeding-type, followed by predators (2B: 20.93 ± 6.21%, 6 genera) and epigrowth-feeders (2A: 19.25 ± 8.7%, 16 genera), while the selective deposit-feeders (1A: 6.93 ±5.08%, 12 genera) contributed with the lowest density (Fig. 7). In all sampling occasions, the same tendency of distribution of feeding types was observed. Regarding the sampling stations, all sites presented a dominance of 1B feeding-type, and selective deposit-feeders were the less abundant; but while the second more important feeding group at the upstream stations was that of predators (2B), the epigrowth feeders predominated in the downstream ones (Fig. 7). PERMANOVA analysis of trophic structure data showed that there were significant differences ($p<0.05$) between sampling occasions and among stations. There was also a significant interaction between both factors (Table 7, see Appendix, Table II).

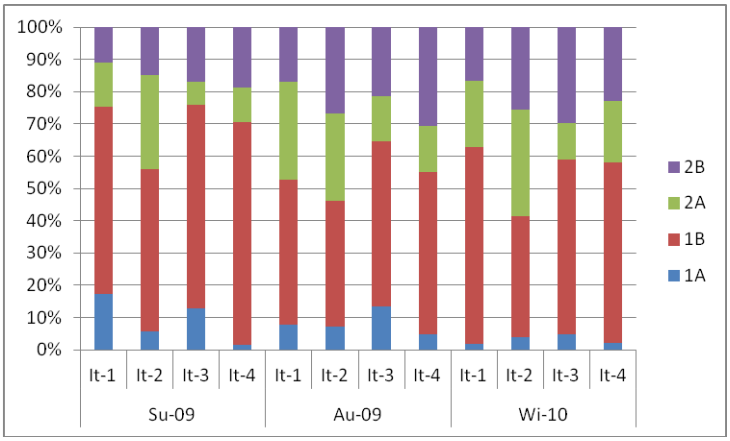

Fig. 7 - Percentage of contribution of the different trophic groups at each sampling station (It-1, It-2, It-3 and It-4) and sampling occasion (summer, Su-09; autumn, Au-09 and winter, Wi-10). 1A – selective deposit feeders; 1B – non-selective deposit feeders; 2A – epigrowth feeders; 2B – omnivores/predators.

Table 7 - Details of the univariate two-factor PERMANOVA test for all nematode descriptors analysed. Bold values stand for the significant differences ($p < 0.05$).

	Source of variation	Degrees of freedom	Pseudo-F	P(perm)
Total density	Sampling occasion	2	23.061	**0.001**
	Station	3	4.3364	**0.007**
	Sampling occasion x Station	6	3.2886	**0.003**
Number of genera	Sampling occasion	2	34.24	**0.001**
	Station	3	12.228	**0.001**
	Sampling occasion x Station	6	1.1173	0.353
Trophic composition	Sampling occasion	2	42.022	**0.001**
	Station	3	4.846	**0.001**
	Sampling occasion x Station	6	3.0579	**0.001**
Margalef Index	Sampling occasion	2	6.5612	**0.003**
	Station	3	17.683	**0.001**
	Sampling occasion x Station	6	1.2285	0.282
Shannon-Wiener Index	Sampling occasion	2	32.632	**0.001**
	Station	3	16.149	**0.001**
	Sampling occasion x Station	6	1.0441	0.403
Trophic Diversity Index	Sampling occasion	2	6.2348	**0.001**
	Station	3	5.1221	**0.007**
	Sampling occasion x Station	6	0.65696	0.710
Maturity Index	Sampling occasion	2	3.3794	**0.034**
	Station	3	12.032	**0.001**
	Sampling occasion x Station	6	1.3917	0.214

3.2.2.3. Indices estimation

The highest total density of nematodes was observed in autumn (Fig. 8A). PERMANOVA confirmed clear temporal differences, and to a less extent spatial ones. It also detected a significant interaction between both factors (Table 6, see Appendix, Table II). Regarding some of the other descriptors, namely the number of genera, and the Margalef and the Shannon-Wiener indices, they were, overall, higher in autumn (Fig. 8B, 8C and 8D, respectively). PERMANOVA revealed differences among sampling occasions and between stations, but no interaction between the two factors (Table 6). In general, individual pair-wise comparisons revealed differences between all sampling occasions except between summer and winter (see Appendix, Table II). Among stations, there were significant differences in genera richness and Margalef Index between all pairs except between It-1 and It-4, and between It-2 and It-3 (see Appendix, Table II), while the Shannon-Wiener Index showed that all pairs of stations differed from each other, except between It-2 and It-3 (see Appendix, Table II). Overall, It-1 and It-4 showed lower diversity (Fig. 8C and 8D).

The Index of Trophic Diversity ranged from 0.34 (It-2, autumn and winter) to 0.52 (It-4, summer) (Fig. 8E). PERMANOVA showed differences between all sampling occasions except between summer and winter (Table, 6; see Appendix, Table II). Significant differences between stations (Table 6) were only observed between It-2 and all other pair of stations (see Appendix, Table II), with lower values in this station, indicating higher trophic diversity (Fig. 8E).

The Maturity Index (MI) ranged between 2.3 (It-4, all sampling occasions; It-1, winter) and 2.7 (It-2, winter) and most nematodes showed a c–p value of 2 (mean, 63%), followed by c–p values of 3 (mean, 31%) (Fig. 8F). PERMANOVA

detected seasonal and spatial significant differences, regarding MI, and no interaction between both factors (Table 6). The seasonal variation was significant only between summer and autumn (see Appendix, Table II). PERMANOVA performed on the Maturity Index revealed no spatial differences only between It-3 and It-1 and It-2 (see Appendix, Table II).

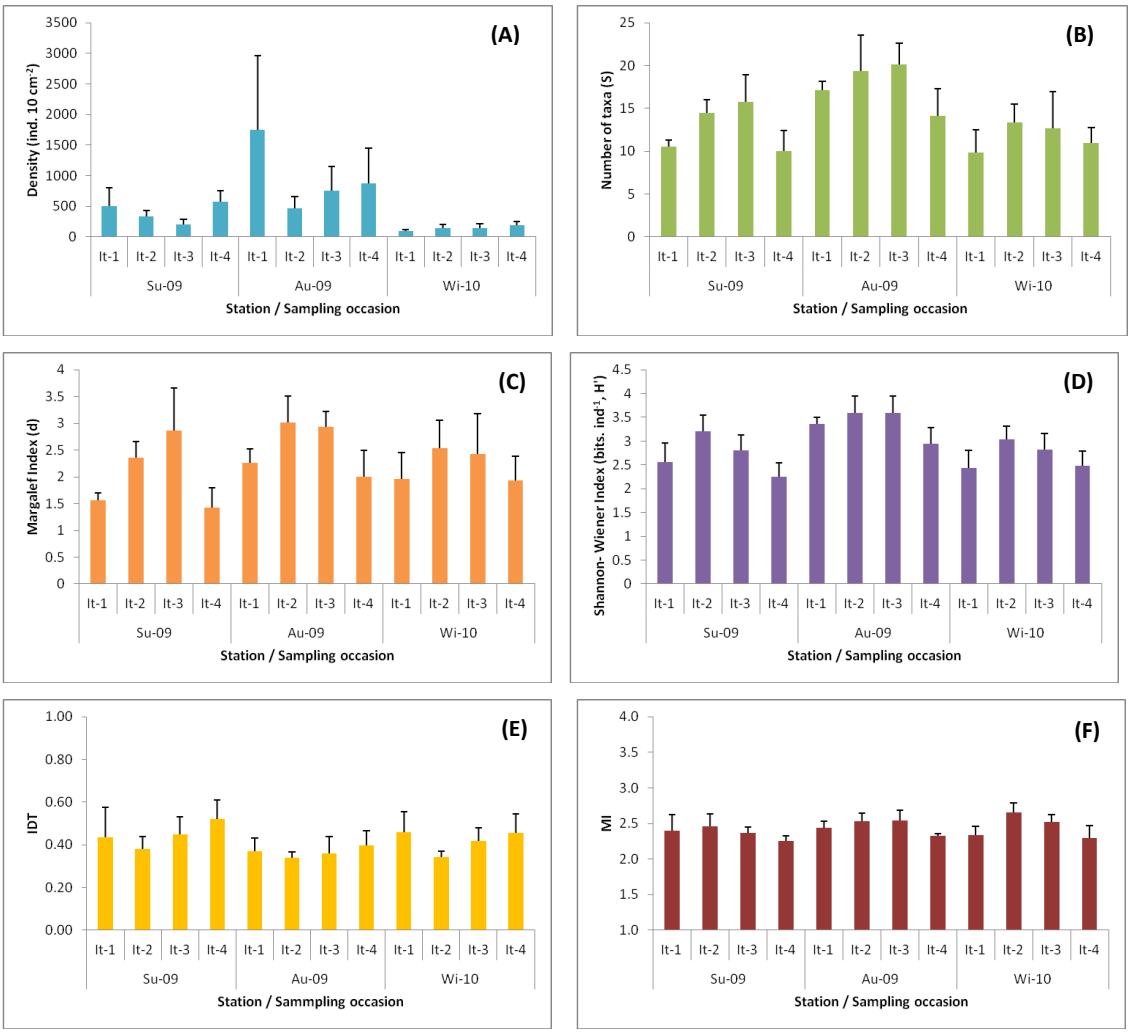

Fig. 8 - Nematode (A) Mean density ± SD (ind. 10 cm^{-2}); (B) Mean number of taxa ± SD; (C) Mean Margalef index ± SD; (D) Mean Shannon-Wiener index (bits. ind^{-1}) ± SD; (E) Mean Index of Trophic Diversity and (F) Mean Maturity Index ± SD, assessed at each sampling station (It-1, It-2, It-3 and It-4), in each sampling occasion (summer, Su-09; autumn, Au-09; winter, Wi-10).

3.3. Relationship between biotic and environmental variables

In order to analyse which factors influenced the distribution of meiofauna and nematodes along the south arm subsystem a Redundance Analysis (RDA) was performed. RDA revealed that the distribution of meiofauna was mainly influenced by phosphates, organic matter, gravel, salinity and dissolved oxygen. The first two RDA ordination axes explained 81.8% of taxa variability and 94.5% of the relationship between abundance and the five selected environmental variables (Table 8A). The high correlation between taxa and environmental parameters obtained for the first two axes (Table 8A) suggests that environmental variables explain adequately the variability associated with taxa abundance. The global permutation test showed that for the first canonical axis (F-ratio=17.148), as well as for the sum of all canonical axes (F-ratio=7.710), relations between taxa abundance and those environmental variables were statistically significant (p=0.002). RDA revealed that the conditions responsible for higher densities of nematodes and nauplii larvae were higher values of organic matter in the sediment and salinity and low phosphate concentrations, whereas the majority of taxa (Copepoda, Polychaeta, Oligochaeta, Gastropoda, Halacaroidea, Cladocera, Bivalvia and Turbellaria) were influenced by higher concentrations of phosphates and dissolved oxygen. Crustacea abundance was linked to an increase in gravel content of sediment (Fig. 9).

In respect to nematodes, RDA showed that the main factors influencing their distribution were phosphates, dissolved oxygen, salinity, ammonium and temperature. The first two ordination axes explained 66.3% of nematode genera abundance variability and 82.3% of the relationship between abundance and environmental variables. The correlation between taxa and environmental

parameters obtained for the first two axes suggests that these variables explain an important part of the variability associated with taxa abundance (Table 8B). The global permutation test showed that the relations between genera abundance and environmental variables were statistically significant ($p<0.05$) for the first canonical axis (*F*-ratio=7.767) as well as for the sum of all canonical axes (*F*-ratio=4.990).

Table 8 - Results of the ordination by RDA performed for (A) meiofauna taxa and (B) nematode genera considering all five explanatory environmental variables selected.

		Axis 1	Axis 2
A. Meiofauna	Eigenvalues :	0.741	0.077
	Taxa/Environment correlations :	0.969	0.817
	Cumulative % variance	74.1	81.8
	Cumulative % variance taxa/environment	85.6	94.5
B. Nematoda	Eigenvalues :	0.564	0.099
	Genera/Environment correlations :	0.963	0.934
	Cumulative % variance	56.4	66.3
	Cumulative % variance Genera/Environment	70	82.3

RDA revealed that the densities of *Sabatieria* were strongly affected by a decrease in phosphates and an increase in salinity; these conditions were also responsible for higher densities of the dominant genera *Daptonema*, *Sphaerolaimus*, *Paralinhomoeus* and *Terschellingia*. An increase in salinity and ammonium content influenced the abundance of *Paracomesoma*, *Spilophorella* and *Calyptronema* at the oppose stations It-1 and It-4, during summer. The less abundant genera *Aponema*, *Hypodonthalaimus*, *Comesoma* and *Bathylaimus* were linked to an increase in water temperature. RDA revealed also that the majority of the other nematode genera were related to an increase in phosphates and dissolved oxygen (Fig. 10).

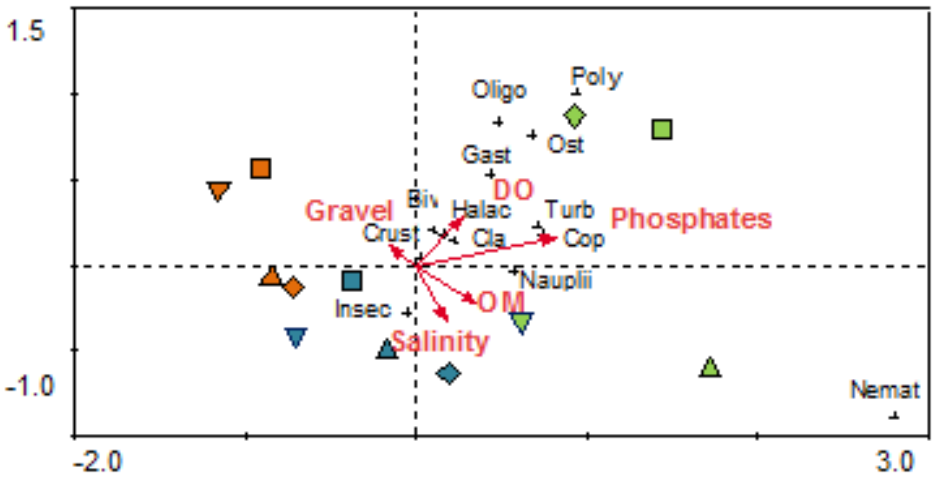

Fig. 9 – Ordination diagram of the RDA relating environmental variables and the distribution of meiofauna in south arm of Mondego estuary. Taxa are represented by crosses (for codes see Table 2). The explanatory environmental variables are indicated by arrows. Sampled stations by symbols (It-1, △; It-2, ▽ ; It-3, □ ; It-4, ◇); sampling occasions by colours (Su-09, ■ ; Au-09, ■ ; Wi-10, ■).

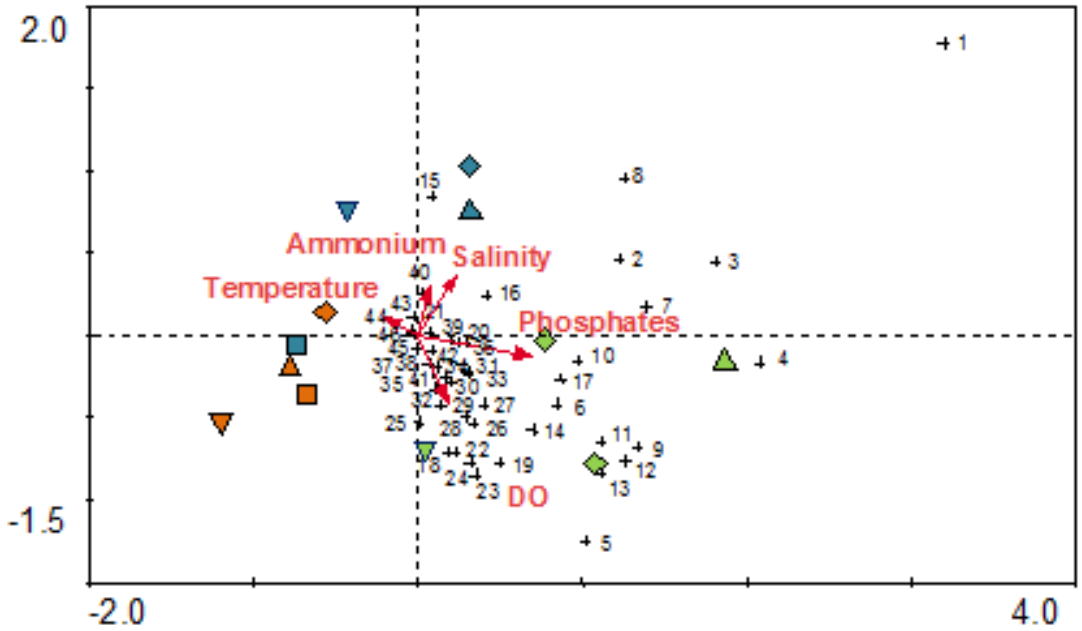

Fig. 10– Ordination diagram of the RDA relating environmental variables and the distribution of nematodes in south arm of Mondego estuary. Genera are represented by crosses (for codes see Table 5). The explanatory environmental variables are indicated by arrows. Sampled stations by symbols (It-1, △ ; It-2, ▽ ; It-3, □ ; It-4, ◇); sampling occasions by colours (Su-09, ■ ; Au-09, ■ ; Wi-10, ■).

4. DISCUSSION

4. DISCUSSION

Extensive sampling on the meiobenthos community in the south arm of the Mondego estuary provided an evaluation of the relationships between meiofauna communities, and nematodes in particular, to their habitat. The results obtained provided a general picture of the spatial distribution of meiofauna communities and nematodes in a relatively restricted area and their seasonal variability. The physicochemical parameters investigated in this study represent some of the ones known to affect the composition and diversity of meiofauna and nematodes (e.g. Higgins &Thiel, 1988; Heip et al., 1995), and were used to characterize estuarine meiobenthos habitats.

The environmental characterization of the Mondego estuary was based on physicochemical parameters recorded simultaneously with meiofauna samples collection, at each sampling event. The characterization of a system based on physicochemical parameters, as they only provide information about the quality at the time of the measurements, lacks the sensitivity to determine the impact of previous events on the ecology of the system (Spellman & Drinan, 2001). The biological communities, especially the sessile benthic organisms, can constitute a sort of memory for the system about past conditions and accurately assess ecological conditions. Meiobenthic communities and more specifically nematodes have several characteristics that make them potentially suitable biological quality indicators, such as high diversity and richness, short life-cycles and limited dispersion ability (Meiofauna: Coull & Chandler, 1992; Coull, 1999; Kennedy & Jacoby, 1999; Nematodes: Boyd et al., 2000; Schratzberger et al., 2002, 2006; Gheskiere et al., 2005).

Since this study was carried out in a relatively restricted area (ca. 3 Km from It-1 to It-4), the variations observed during the survey period were mainly seasonal, rather than spatial. Even so, spatial heterogeneity was also detected in the south arm regarding meiofauna and nematode communities. The strong seasonal variability and the important gradient of environmental conditions along the south arm greatly affected the seasonal and spatial distribution of the meiobenthic and nematode communities within this Mondego estuary subsystem during the studied period.

During the study period the occurrence of extreme climatic events were recorded in the region, namely a severe drought period from March until October, 2009, followed by a period of heavy rain and flooding starting in November, 2009 until April, 2010 (Instituto de Meteorologia, IP, 2009a, 2009b, 2010). Such extreme events changed the environmental characteristics of the estuary, especially salinity. Although, spatially speaking, salinity increased from upstream toward the downstream stations in the south arm, a dramatic seasonal change in salinity values was observed, ranging from 26.9 to 32.8 in summer, to 10.1 to 19.4 in autumn and 5.4 to 8.8 in winter. According to Attrill (2002) and Ferrero et al. (2008), salinity variation over time may be more important than average salinity for the distribution of nematodes along the estuary. Furthermore, the severe flood may have caused sediment displacement and erosion as well as changes in the interstitial water salinity (Santos et al., 1996), causing the dislodgement of organisms and leading to the overall low density values observed during winter. Both salinity and sediment structure are major factors influencing meiobenthic and nematode community structure (e.g. Heip et al., 1985), as it was confirmed in this study. Results from

RDA analysis showed that the distribution patterns of meiofauna and nematodes was mainly structured by distinct environmental factors like nutrients in the water, salinity, dissolved oxygen and sediment grain size, supporting the primary influence of the estuarine gradient on meiobenthic community patterns suggested in other studies (Austen & Warwick, 1989; Vincx et al., 1990; Coull, 1999; Ferrero et al., 2008; Schratzberger et al., 2008; Adão et al., 2009; Alves et al., 2009, 2013; Patricio et al., 2012). The variations in these parameters clearly affected the meiofauna and nematode communities, illustrating the importance of extreme events in changing the characteristics of estuarine ecosystems.

Meiofauna density and diversity were similar to other meiofauna communities, with densities falling within the range observed in other European estuaries (e.g. Soetaert et al., 1995; Rzeznik-Orignac et al., 2003). The dominance of nematodes over all other taxa is well documented, with Nematoda typically being the most abundant taxon (usually 60–90%) (Coull, 1999). Overall, Polychaeta ranked second, closely followed by copepods. In fact, scrutinizing the meiofaunal data, only in autumn Polychaeta were more abundant than copepods, and even then if the copepods with nauplius larvae stages totals were considered together, densities in the latter broad group were higher. These results are in general agreement with literature, where it is a common observation that copepods are usually the second more abundant taxon (e.g. Coull, 1999; Rzeznik-Orignac et al., 2003).

In temperate regions, nematode densities usually peak in the warmest months (Hicks and Coull, 1983; Smol et al., 1994) but in this study the highest density was observed in autumn. The same was observed in the intertidal

meiofaunal communities of the Mira estuary (Adão, 2003). The influence of physical factors might partly explain the summer decrease in nematode density in Mondego south arm. As pointed out by Guarini et al. (1997), temperature and salinity of emerged sediments are more extreme during summer tidal cycles, and that phenomenon was observed in this region during this season. The circumstance of a long drought period during the spring and summer of 2009 can explain these results: autumn conditions were more favorable for meiofauna and nematode communities than summer during this year. One more example showing that the estuarine abiotic gradient was mostly similarly reflected in the distribution patterns of the biological communities of Mondego estuary south arm.

In the present study, the analysis of spatial and temporal structural variations in meiofaunal and nematode communities provided essentially similar results. Results from the MDS analysis carried out using meiofauna taxa as the input variables (Fig. 3), showed a clear separation between sampling occasions, and to a lesser extent, between sampling stations. Interestingly, the same analysis carried out using the data on nematode genera (Fig. 6) displayed the same distribution pattern. This suggests that both approaches are effective in capturing changes in meiofauna community structure over this local scale. Furthermore, RDA analysis for both communities showed that three of the main environment variables explaining each distribution patterns were common, namely phosphates, dissolved oxygen and salinity. Other studies of meiofauna communities have shown that meiofauna taxa assemblages could provide a sensitive and clear measure of environmental status (marine environments: Schratzberger et al., 2000; harbours: Moreno et al., 2008).

Taking into account the meiobenthic and nematode assemblages over space and time, the meiofauna and nematodes composition appeared similar, according to the SIMPER results (Tables 3 and 7, respectively). Nevertheless, to some extent, it was possible to recognize the occurrence of spatial and temporal heterogeneity. Not surprisingly, when comparing both communities, it is apparent that nematode assemblages presented higher contribution in the dissimilarities at the spatial (24.6 - 34% *vs.* 42.1 - 47.7%) and temporal (35.2 - 55.5% *vs.* 47.6 - 57.2%) scales. Since the more abundant taxa of meiofauna is Nematoda, increasing taxonomic resolution (from phyllum to genus level) of this taxon allows to enhance the knowledge of the system under study in further detail and consequently discriminate different communities easier.

Nematodes communities comprised a high number of genera but with few dominant ones, as observed in other estuaries (e.g. Warwick, 1971; Austen et al., 1989; Li & Vincx, 1993; Soetaert et al., 1995; Rzeznik- Orignac et al., 2003; Steyaert et al., 2003; Ferrero et al., 2008). *Sabatieria*, *Daptonema* and *Terschellingia*, three of the most abundant genera in the present study, are known to be tolerant to pollution (Soetaert et al., 1995; Austen & Somerfield, 1997; Schratzberger et al., 2006; Steyaert et al., 2007; Gambi et al., 2009; Armenteros et al., 2009), and their high densities along the south arm of the Mondego estuary may be indicative of the pressures from which this estuarine subsystem suffers.

In order to reduce community data into one or a few variables, simplifying its analysis, interpretation or review, several ecological indices have been suggested (Salas, 2006; Neher & Darby, 2009). Coupled with the taxonomic diversity, functional diversity is important in interpreting distribution patterns of

the communities (Schratzberger et al., 2008). Regarding meiobenthic communities, besides the common abundance and diversity measures, specific indicators rely on nematodes information, such as the Maturity Index and the Index of Trophic Diversity, as well as the Nematode/copepod index, at a broader resolution. These three indices do not depend on the system, and thus do not suffer from lack of generality, and the use of indicators based on different ecological principles is subsequently highly recommended (Dauer et al., 1993) in determining the environmental quality status of an ecosystem (Marques et al., 2009). Since the south arm of the Mondego estuary is known to suffer from anthropogenic pressures, especially inputs from the Pranto River and agricultural run-off, it is important to evaluate the performance of the indices in differentiating sectors of impact along the estuary. The results suggest that both abundance and all diversity indices studied for meiofauna assemblages were higher in autumn and lower in winter. Spatially, It-1 was the station that revealed higher density, but it was station It-3 that presented higher taxa richness and diversity (Margalef and Shannon-Wiener indices). In respect to Nematode/copepod index, accepting the premise that Harpacticoid copepods are sensitive to environmental perturbation (Hicks & Coull, 1983; Van Damme et al., 1984; Stoetaert et al., 1995) and therefore low densities may indicate anthropogenic disturbances, it was observed that in the south arm of the Mondego estuary the only station presenting values higher than 100 (mean value: 114), was It-1, during summer; all other stations revealed values under 50, which suggests a low organic polluted ecosystem. The application of the indices for nematode communities revealed similar results. With regard to sampling occasions, autumn showed again to be the season with higher values

of density, genus richness and taxonomic diversity. Spatially, It-1 was the station characterized by higher nematode density, and It-3 the station richer in genera and with higher Margalef diversity, while Shannon-Wiener diversity was higher at It-2. Nevertheless, there were no significant statistical differences between the two stations (It-3 and It-2), regarding values of both indices. Interestingly, station It-1 was always the richer station with respect to organic matter content and the one with the largest silt-clay fraction in the sediment. As for It-3, it presented always the lower content in organic matter, and its sediment is characterized by higher percentages of fine to mean sand. These results are in accordance with the observation that nematodes density tend to increase in muddy sediments, while the diversity increases in sandy sediments (Heip et al., 1985), probably due to the wider range of microhabitats available in sandy bottoms as compared to muddy ones (Steyaert et al., 2003). With respect to nematode functional indices: Trophic Diversity index, generally used to correlate trophic diversity with pollution levels (Heip et al., 1985), and Maturity Index, which low values suggests a high stress level, since opportunistic genera increase in abundance in adverse conditions (Bongers & Bongers, 1998; Gyedu-Ababio & Baird, 2006), the results verified that the indices behaved in agreement. For example, Trophic Diversity index was higher during summer, at It-4, when Maturity index presented its lower value, whereas in autumn, in It-2, the reverse occurred, revealing a relatively better ecological condition in It-2 in autumn as compared to station It-4 in summer. These results are in agreement with other works carried out in Mondego (Patrício et al., 2012; Alves et al., 2013), and were somewhat to be expected as It-4 receives inputs from the Pranto River and agricultural runoffs, some of the main anthropogenic

pressures known for this subsystem. However, a better knowledge of sources of pollution and anthropogenic pressures at each sampling station of the south arm would be desirable to interpret these results more accurately.

5. CONCLUDING REMARKS

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The present study combined temporal and spatial information on meiofauna and nematodes in particular in the south arm of Mondego estuary, allowing a full description of the intertidal meiobenthic communities along this estuarine subsystem. This study was the first to investigate the meiofauna intertidal communities of the Mondego estuary and can complement knowledge about meiofauna and nematode communities in this ecosystem. Information made available reinforced the preliminary meiofauna baseline previously carried out in the Mondego estuary.

This work demonstrated that the estuarine abiotic gradient was mostly similarly reflected in the distribution patterns of the biological communities of Mondego estuary south arm, being the temporal variation more important than the spatial one. Both biological communities studied were mainly influenced by the concentration of phosphates, salinity and dissolved oxygen.

This study also illustrated that data on spatial and seasonal distribution patterns of intertidal meiofauna and nematode communities in particular in the Mondego estuary, namely during a sequence of extreme climatic events, provided essentially the same ecological information. Besides, results obtained in the south arm of the Mondego estuary suggest that meiofauna taxa assemblages may provide a sensitive tool for environmental conditions assessment.

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ANNEX

Table I – Classification of nematode trophic groups according to Wieser (1953).

Trophic group		Criteria	Classification
Group I (without buccal armature)	1A	Species without a buccal cavity or with a narrow tubular buccal cavity	selective deposit feeders
	1B	Species with a large buccal cavity	non-selective feeders
Group II (with buccal armature)	2A	Species with a buccal cavity armed with small or moderate sized teeth	epigrowth or diatom feeders
	2B	Species with large teeth or jaws	Preators or Ominvores

Table II – Families, feeding types and c-p value of all genera found in the four stations during the three sampling occasions.

Genus	Family	Feeding type	c-p value	Genus	Family	Feeding type	c-p value
Antomicron (Cobb, 1920)	Leptolaimidae	1A	2	*Linhomoeus* (Bastian, 1865)	Linhomoeidae	2A	4
Aegialoalaimus (De Man, 1907)	Aegialoalaimidae	1A	4	*Metachromadora* (Filipjev, 1918)	Desmodoridae	2B	2
Anoplostoma (Bütschli, 1874)	Anoplostomatidae	1B	3	*Metalinhomoeus* (De Man, 1907)	Linhomoeidae	1B	2
Aponema (Jensen, 1978)	Microlaimidae	1A	3	*Microlaimus* (De Man, 1880)	Microlaimidae	2A	2
Araeolaimus (De Man, 1888)	Diplopeltidae	1A	3	*Molgolaimus* (Ditlevsen, 1921)	Desmodoridae	1A	4
Axonolaimus (De Man, 1889)	Axonolaimidae	1B	3	*Nemanema* (Cobb, 1920)	Oxystominidae	1A	3
Bathylaimus (Cobb, 1893)	Tripyloididae	1B	3	*Odontophora* (Bütschli, 1874)	Axonolaimidae	1B	4
Calyptronema (Marion, 1870)	Enchelidiidae	2B	3	*Oncholaimellus* (De Man, 1890)	Oncholaimidae	2B	3
Camacolaimus (De Man, 1889)	Leptolaimidae	2A	2	*Oncholaimus* (Dujardin, 1845)	Oncholaimidae	2B	4
Chromadora (Bastian, 1865)	Chromadoridae	2A	2	*Oxystomina* (Filipjev, 1921)	Oxystominidae	1A	3
Chromadorella (Filipjev, 1918/21)	Chromadoridae	2A	2	*Paracanthonchus* (Micoletzky, 1924)	Cyatholaimidae	2A	3
Chromadorina (Filipjev, 1918/21)	Chromadoridae	2A	3	*Paracomesoma* (Hope & Murphy, 1972)	Comesomatidae	2A	2
Chromadorita (Filipjev, 1922)	Chromadoridae	2A	4	*Paracyatholaimus* (Micoletzky, 1922)	Cyatholaimidae	2A	2
Comesoma (Bastian, 1865)	Comesomatidae	1B	3	*Paralinhomoeus* (De Man, 1907)	Linhomoeidae	1B	4
Cyartonema (Cobb, 1920)	Aegialoalaimidae	1A	3	*Prochromadorella* (Micoletzky, 1924)	Chromadoridae	2A	2
Cyatholaimus (Bastian, 1865)	Cyatholaimidae	2A	2	*Ptycholaimellus* (Cobb, 1920)	Chromadoridae	2A	3
Daptonema (Cobb, 1920)	Xyalidae	1B	2	*Sabatieria* (Rouville, 1903)	Comesomatidae	1B	2
Desmolaimus (De Man, 1880)	Linhomoeidae	1B	2	*Sphaerolaimus* (Bastian, 1865)	Sphaerolaimidae	2B	2
Dichromadora (Kreis, 1929)	Chromadoridae	2A	3	*Spilophorella* (Filipjev, 1918/21)	Chromadoridae	2A	2
Eleutherolaimus (Filipjev, 1922)	Linhomoeidae	1B	2	*Terschellingia* (De Man, 1888)	Linhomoeidae	1A	3
Halalaimus (De Man, 1888)	Oxystominidae	1A	2	*Thalassoalaimus* (De Man, 1893)	Oxystominidae	1A	3
Hypodontolaimus (De Man, 1886)	Chromadoridae	2A	4	*Theristus* (Bastian, 1865)	Xyalidae	1B	2
Leptolaimus (De Man, 1876)	Leptolaimidae	1A	2	*Viscosia* (De Man, 1890)	Oncholaimidae	2B	3

APPENDIX

Table I – Results of pair-wise comparisons tests of the two-factor PERMANOVA for all meiofauna descriptors analyzed. Bold values stand for the significant differences ($p < 0.05$).

		Term: Sampling occasion		Term: Sampling occasion x Station Factor: Sampling station									Term: Station		Term: Sampling occasion x Station Factor: Station					
				It-1		It-2		It-3		It-4					Su-09		Au-09		Wi-10	
	Gropus	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	Groups	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
Total density	**Su-09, Au-09**			2.7987	**0.013**	2.2492	0.05	4.7889	**0.007**	1.5487	0.142	**It-1, It-2**			1.3085	0.224	2.5033	**0.032**	1.9133	0.08
	Su-09, Wi-10			3.3611	**0.007**	3.8427	**0.007**	0.17673	0.889	5.3215	**0.004**	**It-1, It-3**			2.0491	**0.04**	1.4377	0.22	2.163	0.06
	Au-09, Wi-10			3.7211	**0.002**	3.6529	**0.009**	5.2633	**0.004**	3.1601	**0.004**	**It-1, It-4**			0.53588	0.63	1.4765	0.178	3.469	**0.009**
												It-2, It-3			2.0687	0.07	2.3698	**0.04**	8.99E-01	0.381
												It-2, It-4			3.0154	**0.024**	1.3941	0.209	0.8711	0.411
												It-3, It-4			4.1107	**0.02**	0.26028	0.775	0.35209	0.754
Number of taxa	**Su-09, Au-09**	8.0711	**0.001**									**It-1, It-2**	1.1198	**0.287**						
	Su-09, Wi-10	6.9455	0.001									**It-1, It-3**	3.2891	**0.003**						
	Au-09, Wi-10	13.395	**0.001**									**It-1, It-4**	0.078943	0.934						
												It-2, It-3	2.5266	0.022						
												It-2, It-4	1.5094	**0.129**						
												It-3, It-4	4.4497	**0.001**						
Margalef Index	**Su-09, Au-09**			0.73382	0.521	4.5342	**0.004**	1.9712	0.067	5.9885	**0.002**	**It-1, It-2**			0.30177	0.75	2.1843	0.054	1.2559	0.214
	Su-09, Wi-10			3.7406	**0.004**	3.1935	**0.018**	0.87264	0.424	1.6436	0.152	**It-1, It-3**			1.5598	0.156	2.5066	**0.031**	4.0416	**0.003**
	Au-09, Wi-10			2.8738	**0.014**	6.941	**0.003**	4.1467	**0.003**	11.295	**0.002**	**It-1, It-4**			2.5392	0.041	2.0361	0.076	0.82601	0.426
												It-2, It-3			1.3448	0.205	0.46694	0.657	3.6155	**0.007**
												It-2, It-4			2.5407	**0.041**	0.51155	0.565	2.7503	**0.023**
												It-3, It-4			2.7098	**0.048**	1.0603	0.276	6.314	**0.005**
Shannon-Wiener Index	**Su-09, Au-09**			2.0749	**0.031**	4.3568	**0.007**	4.285	**0.004**	8.1696	**0.003**	**It-1, It-2**			1.3196	0.229	2.4905	**0.04**	0.20155	0.836
	Su-09, Wi-10			3.3113	**0.018**	0.51304	0.607	1.76	0.134	1.63	0.145	**It-1, It-3**			5.6991	**0.005**	3.9938	**0.006**	3.5009	**0.008**
	Au-09, Wi-10			0.58775	0.66	3.4722	**0.018**	2.6715	**0.032**	11.101	**0.004**	**It-1, It-4**			0.4546	0.656	2.1722	0.051	4.6118	**0.008**
												It-2, It-3			2.6482	**0.046**	1.6822	0.11	2.8413	**0.023**
												It-2, It-4			0.91741	0.379	1.0417	0.347	2.1904	**0.024**
												It-3, It-4			4.8146	**0.014**	3.1026	**0.014**	6.2628	**0.002**
Nematode/Copepod Ratio	**Su-09, Au-09**	2.5166	**0.01**									**It-1, It-2**								
	Su-09, Wi-10	2.2168	0.033									**It-1, It-3**								
	Au-09, Wi-10	0.14375	0.859									**It-1, It-4**								
												It-2, It-3								
												It-2, It-4								
												It-3, It-4								

Table II – Results of pair-wise comparisons tests of the two-factor PERMANOVA for all nematodes descriptors analysed. Bold values stand for the significant differences ($p < 0.05$).

		Term: Sampling occasion		Term: Sampling occasion x Station Factor: Sampling station									Term: Station		Term: Sampling occasion x Station Factor: Station					
				It-1		It-2		It-3		It-4					Su-09		Au-09		Wi-10	
	Gropus	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	Groups	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
Total density	**Su-09, Au-09**			2.4591	**0.025**	1.5375	0.172	3.0405	**0.008**	1.1122	0.378	**It-1, It-2**			1.3308	0.213	2.5776	**0.017**	1.8756	0.054
	Su-09, Wi-10			3.3362	**0.005**	3.7769	**0.007**	1.2798	0.252	5.0477	**0.005**	**It-1, It-3**			2.1165	**0.023**	1.9177	0.095	1.7264	0.128
	Au-09, Wi-10			3.378	**0.002**	3.9171	**0.005**	3.7347	**0.004**	2.9173	**0.005**	**It-1, It-4**			0.47915	0.653	1.6029	0.166	3.8129	**0.006**
												It-2, It-3			2.153	0.057	1.6191	0.099	5.74E-02	0.968
												It-2, It-4			2.8863	**0.026**	1.6578	0.152	1.3287	0.215
												It-3, It-4			4.2135	**0.02**	0.41872	0.706	1.165	0.28
Number of genera	**Su-09, Au-09**	6.6059	**0.001**									**It-1, It-2**	4.1177	**0.001**						
	Su-09, Wi-10	1.3195	0.184									**It-1, It-3**	4.108	**0.001**						
	Au-09, Wi-10	7.1361	**0.001**									**It-1, It-4**	1.0759	0.296						
												It-2, It-3	0.46014	0.616						
												It-2, It-4	4.3764	**0.001**						
												It-3, It-4	4.3481	**0.002**						
Trophic composition	**Su-09, Au-09**			2.6682	**0.021**	1.1943	0.228	4.512	**0.005**	1.4949	0.15	**It-1, It-2**			1.5156	0.11	2.4946	**0.013**	2.2501	**0.005**
	Su-09, Wi-10			4.8127	**0.003**	2.942	**0.004**	1.2215	0.212	3.5408	**0.003**	**It-1, It-3**			2.4858	**0.022**	1.7699	0.093	1.2169	0.234
	Au-09, Wi-10			6.3069	**0.005**	3.0021	**0.012**	3.8208	**0.006**	4.0305	**0.005**	**It-1, It-4**			1.6578	0.1	1.5713	0.126	2.7226	**0.001**
												It-2, It-3			2.6915	**0.004**	1.571	0.078	1.19E+00	0.247
												It-2, It-4			2.6242	**0.007**	1.4411	0.163	2.0382	**0.041**
												It-3, It-4			3.9473	**0.01**	0.75171	0.627	0.952	0.507
Margalef Index	**Su-09, Au-09**	3.9813	**0.001**									**It-1, It-2**	5.4258	**0.001**						
	Su-09, Wi-10	1.0827	0.32									**It-1, It-3**	4.6957	**0.001**						
	Au-09, Wi-10	2.3632	**0.03**									**It-1, It-4**	1.0272	0.334						
												It-2, It-3	0.52749	0.603						
												It-2, It-4	5.6253	**0.001**						
												It-3, It-4	4.9581	**0.001**						
Shannon-Wiener Index	**Su-09, Au-09**	6.811	**0.001**									**It-1, It-2**	4.4933	**0.001**						
	Su-09, Wi-10	5.31E-02	0.962									**It-1, It-3**	2.5411	**0.016**						
	Au-09, Wi-10	7.2736	**0.001**									**It-1, It-4**	2.114	**0.044**						
												It-2, It-3	1.8075	0.085						
												It-2, It-4	6.5189	**0.001**						
												It-3, It-4	4.5373	**0.001**						
Trophic Diversity Index	**Su-09, Au-09**	3.3294	**0.006**									**It-1, It-2**	2.6306	**0.008**						
	Su-09, Wi-10	1.1721	0.259									**It-1, It-3**	0.44611	0.666						
	Au-09, Wi-10	2.6268	**0.012**									**It-1, It-4**	1.0239	0.324						
												It-2, It-3	2.7346	**0.015**						
												It-2, It-4	4.7543	**0.001**						
												It-3, It-4	1.7209	0.093						
Maturity Index	**Su-09, Au-09**	2.3865	**0.02**									**It-1, It-2**	3.0055	**0.008**						
	Su-09, Wi-10	1.8959	0.081									**It-1, It-3**	1.7991	0.09						
	Au-09, Wi-10	0.34402	0.737									**It-1, It-4**	2.2664	**0.039**						
												It-2, It-3	1.548	0.119						
												It-2, It-4	5.9547	**0.001**						
												It-3, It-4	5.0811	**0.001**						