



**SPATIAL DISTRIBUTION OF THE SUBTIDAL MEIOFAUNA
COMMUNITIES ALONG A SALINITY GRADIENT IN TWO
SOUTHERN EUROPEAN ESTUARIES**

Distribuição espacial das comunidades subtidais de meiofauna ao longo do
gradiente de salinidade em dois estuários do Sul da Europa



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"Esta dissertação não inclui as críticas feitas pelo júri"

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**SPATIAL DISTRIBUTION OF THE SUBTIDAL MEIOFAUNA COMMUNITIES
ALONG A SALINITY GRADIENT IN TWO SOUTHERN EUROPEAN ESTUARIES.**

Abstract

The spatial variations in density and composition of subtidal meiofauna and free-living Nematoda communities were analysed in two Portuguese estuaries, Mira (southwestern coast) and Mondego (western coast), that are subjected to different anthropogenic pressures, along the salinity gradients (from <0.5 to >30psu).

In both estuaries, meiofauna communities were characterised by the dominance of the taxa Nematoda, Copepoda and Polychaeta. The spatial patterns of density and composition of both meiofauna and Nematoda communities reflected the salinity gradient, being these assemblages structured and influenced by this natural stressor. Besides salinity, sediment properties also influenced the communities and the responses of the communities to both anthropogenic and natural stress could not be easily differentiated. Nevertheless, different patterns of the trophic nematode structure assemblages between Mira and Mondego overlapped the salinity effects and the feeding guilds and their response could detect the anthropogenical-induced stress in these estuaries.

Resumo

A variabilidade espacial de densidade e composição de comunidades subtidais de meiofauna e nemátodes de vida livre foi estudada em dois estuários portugueses, Mira (costa sudoeste) e Mondego (costa oeste), sujeitos a diferentes pressões antropogénicas, ao longo do gradiente de salinidade (de <0.5 até >30psu).

Em ambos os estuários, os taxa Nematoda, Copepoda e Polychaeta dominaram as comunidades de meiofauna. Os padrões espaciais de densidade e composição das comunidades de meiofauna e nemátodes reflectiram o gradiente de salinidade, sendo influenciados por este factor de stress natural. Para além da salinidade, as propriedades do sedimento também influenciaram as comunidades e a diferença entre as respostas das comunidades ao stress antropogénico e natural não foram distinguidas. No entanto, a diferente composição trófica das comunidades de nemátodes do Mira e Mondego está relacionada com a diferente pressão antropogénica sentida neste dois sistemas, que se sobrepôs ao efeito da salinidade, tendo sido possível detectar o efeito do stress antropogénico nestes estuários.

Summary

Meiobenthos is an important benthic component of marine and estuarine sediments. In estuarine sediments meiofauna facilitates biomineralization of organic matter, enhancing nutrient regeneration, serves as food for a variety of higher trophic levels and exhibits high sensitivity to environmental modification. Spatial (horizontal and vertical) variations, temporal changes, abundance, species composition and fluctuations of estuarine meiofauna communities are influenced by several biotic and abiotic factors such as trophic relationships, bioturbation, oxygen, salinity, temperature and sediment grain size characteristics.

The objective of this study was to analyse and compare the spatial distribution of the density and composition of subtidal Meiofauna and Nematoda communities in two southern European estuaries, exposed to different degrees of anthropogenic stress, in Portugal: Mira, a relatively undisturbed estuary, and Mondego, a system under severe anthropogenic impacts. Samples were collected along the salinity gradient of the two estuaries, from freshwater (<0.5psu) to euhaline areas (>30psu). Data were analysed in a way to describe and compare the distribution patterns of composition and density of meiofauna taxa and Nematoda communities along the salinity gradients of both estuaries and to identify the specific environmental factors structuring that distribution.

The results have shown that the features of Mira and Mondego estuaries were different concerning sediment properties, with the Mira estuary presenting higher proportions of silt + clay and organic matter content and the Mondego estuary presenting higher percentage of dissolved oxygen and phosphate concentration.

In both estuaries, the meiofauna communities showed a dominance of the Nematoda taxon, followed by Harpacticoid Copepods and Polychaeta. The densities were in general higher in the Mira estuary; 12 higher meiofauna taxa were identified in the Mira estuary and 13 in the Mondego. The comparison of equivalent salinity stretches from the two estuaries revealed significant differences between them and the spatial patterns regarding meiofauna abundance and taxa composition reflected the salinity gradients, with increasing densities from freshwater to euhaline areas. Three distinct assemblages were identified, in accordance with the salinity ranges: Freshwater areas, presenting the lowest meiofauna densities; Oligohaline and Mesohaline areas, presenting low densities and low taxa diversity; and Polyhaline and Euhaline areas, characterised by the highest meiofauna density and high diversity.

The Nematoda genera identified resembled those of the Northern Europe. As the meiofauna taxa, Nematoda densities in the Mira estuary were higher than in the

Mondego, although in the Mondego genera diversity was higher. The nematode genera density and composition presented three distinct assemblages, following the salinity gradients. Densities and genera diversity increased from freshwater to seaward areas and differences between the two estuaries, based on genera composition and density, were difficult to detect although the analyses of the proportion of the trophic guilds of the two estuaries registered differences between them.

Salinity was an important factor in structuring both spatial distribution of meiofauna taxa and Nematoda communities in the studied estuaries, even though other environmental factors, such as granulometry, nutrients concentration and sediment organic matter, had some importance in structuring the communities, overlapping the salinity effects, and creating "site structures" consisting of a complex set of both biotic and abiotic factors. Heterogeneity was much higher along the estuarine gradients than between estuaries, indicating that mesoscale variability within estuaries (at the scale of km) was more important than variability at the scale of hundreds of km or between estuaries. Spatial variability was associated to natural stressors characteristics of estuaries, as salinity and sediment gradients, and the anthropogenic stressors of the Mondego estuary seemed to be not relevant to nematode community density and composition patterns. Nevertheless, different patterns of the trophic structure assemblages between Mira and Mondego overlapped the salinity effects (natural stressor) and the feeding guilds and their response could detect the anthropogenic-induced stress in the studied estuaries.

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Chapter 1

GENERAL INTRODUCTION

Scope of the work

Estuaries are within the most productive systems in the world and represent transitional areas between freshwater and marine ecosystems (Valiela 1995; Kennish, 2000), showing large fluctuations of environmental factors such as tides, salinity patterns, temperature and oxygen, over both different time (from a tidal cycle to a year or longer) and spatial scales. Consequently, the ecology of estuaries is very different from the adjacent freshwater and coastal systems and the complex physical and chemical changes in the estuary impose physiological limits on organisms (McLusky and Elliot, 2004). Benthic organisms, being bound to the sediment, have to adapt to a range of these conditions and their present occurrence is based on a past set of environmental conditions (Soetaert et al., 1995).

The meiobenthos (=meiofauna) consists of small animals with phylogenetic representation from almost all the invertebrate phyla and can be defined as an assemblage of small benthic metazoans having a coherent set of life-history and feeding characteristics which sets them apart as a separate evolutionary unit from larger macrofauna (Warwick, 1984). On a methodological basis, is defined by size based on the standard mesh width of sieves with 500 - 1000 μm as upper and 32 - 63 μm as lower limit. Due to their small size, high abundance (usually in the range of 10^5 to 10^7 ind. m^{-2}), high diversity, short generation times (Heip et al., 1985; Coull, 1999) and high production, usually equal to or higher than that of macrofauna in estuaries, meiobenthos plays an important role in marine and estuarine benthic food chains (Gee, 1989). In fact, its contribution is of extreme importance in nutrient recycling in these waters (Gee, 1989; Coull, 1990) and in linking primary producers and higher trophic levels.

The most numerically important phylum of the meiobenthos in nearshore marine and estuarine waters is the Nematoda, whose individuals often outnumber those of all other meiofaunal taxa collectively (Heip et al., 1985; Coull, 1999), comprising 60-90% of the total meiofauna. Copepods are typically second at 10-40% (Coul 1999) and occasionally a taxon other than nematodes predominates (e.g. Turbellaria, Alongi 1987, 1989; Coull, 1999).

In Europe, meiobenthos and Nematoda studies are almost restricted to the northern European estuaries and studies on the influence of environmental variables over meiobenthic communities allowed the recognition of sediment grain size as a primary factor affecting the abundance of meiobenthic organisms (Warwick, 1971, Coul, 1988, Smol et al., 1994, Heip et al., 1995, Steyaert et al., 2003), as well as

organic content (Warwick, 1971; Moens et al., 1999), extent of oxygenation of the sediment (Coull, 1988) and salinity as the most important physical factors in explaining and controlling meiofaunal abundance and species composition (Soetaert et al., 1995; Coull, 1999). However, since the ecological factors of salinity and substrate are closely interwoven, the recognition of one single factor in explaining the distribution of estuarine organisms is very difficult and, in most estuaries, there is a close connection between salinity distribution and substrate type, with reduced salinity associated with coarser substrates, often making it difficult to distinguish their effects.

It has also been shown that nematodes and copepods can be significantly different at sites as close to each other as several metres as they can at sites kilometres apart, and nematodes generally have aggregated distributions on a scale of centimetres. Physical factors may be more important in generating macro-scale heterogeneity than in generating micro-scale heterogeneity. So, micro-scale changes in the meiofauna spatial distribution can be related to the aggregation of individuals, caused by patchy food distribution and by social or reproductive behaviour (Li et al., 1997) while meso-scale variability (in order of kilometres), due the salinity changes or grain-size differences, is more important than a scale variability of hundreds of kilometres among estuaries (Soetaert et al., 1995).

Despite their importance, there are far less data on diversity and ecology of the meiofauna than the macrofauna, mainly reflecting a bias towards studying organisms that are sufficiently large for ready identification (Giere, 1993; Coull, 1999) and most of the studies performed in European estuaries are referent to intertidal areas (for example Phillips & Fleeger, 1985; Soetaert et al., 1995; Steyaert et al., 2003), while few studies report to subtidal areas (Smol et al., 1994, Soetaert et al., 1994).

Although investigation on the distribution of meiobenthos at different spatial scales is vast (Mokievsky et al., 2004) and the effect of salinity as an important factor structuring meiofauna communities has already been referred, knowledge regarding the influence of the salinity gradient on the meiobenthos community is rather fragmentary (Udalov et al., 2005). Furthermore, with regard to intertidal meiofauna and nematode communities, a number of studies have been performed within narrow salinity ranges (Austen & Warwick, 1989, Capstick, 1959; Warwick & Gee, 1984) while studies on the spatial distribution of subtidal estuarine nematodes along the salinity gradient are very scarce.

The importance of meiofauna as environmental indicators has been recognized from last century (Coull and Chandler, 1992; Kennedy & Jacoby, 1999), especially on the study of the effects of anthropogenic activities and pollution on meiofauna and nematodes recently (Boyd et al., 2000; Schratzberger et al., 2002, 2006; Gheskiere et

al., 2005) since they combine a high diversity with short generation times, responding rapidly to alteration in food supply. Due to their reproductive strategies, changes in population structure usually can be related to a change in environmental conditions (Urban-Malinga et al., 2006).

Objectives

In the present study the spatial variations and composition of subtidal meiobenthos and Nematoda communities were investigated along the salinity gradients of two Portuguese estuaries, Mira and Mondego, which are subjected to different anthropogenic pressures. The aim of the present work is to study the spatial distribution of subtidal meiobenthos and Nematoda communities and to identify the specific environmental factors structuring that distribution through the specific objectives:

- to describe and compare the distribution patterns of Meiobenthos and Nematoda composition and density in subtidal sediments along the salinity gradients of both estuaries;
- to analyse the main environmental variables influencing the structure and distribution of both Meiobenthos and Nematoda assemblages;
- to compare the distribution patterns of the composition and density of Meiobenthos and Nematoda communities between the two estuaries that are subjected to distinct anthropogenic pressures.

The following questions were addressed:

- 1- Do the Meiobenthos and Nematoda composition and density patterns follow the salinity gradient?
- 2- Do the distinct anthropogenic pressures observed in the two estuaries cause significant differences in the distribution of composition and density of the Meiobenthos and Nematoda patterns?

Chapter 2

**Spatial distribution of subtidal meiofauna communities along a salinity gradient
in two Southern European estuaries (Portugal)**

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ABSTRACT

The spatial variation in abundance and composition of subtidal meiofauna communities was studied at two Portuguese estuaries, along a salinity gradient from freshwater (<0.5 psu) to euhaline areas (>30 psu). Samples were collected at 20 sampling stations, of which 11 in the Mondego estuary, a system under severe anthropogenic impact, and 9 in the relatively undisturbed Mira estuary.

Nematoda populations appeared strongly dominant in the meiobenthic communities of both estuaries, although densities were in general higher in the Mira. A total of 12 taxa were recorded in the Mira, of which Harpacticoid copepods and Polychaeta were the second and third most abundant groups, respectively. Of the 13 taxa recorded in the Mondego estuary, Polychaeta was the second most abundant group and Harpacticoid copepods the third.

The comparison of equivalent salinity stretches at both estuaries showed significant differences in meiofauna densities. Total meiofauna, Nematoda, Copepoda, and Polychaeta densities were higher in the Mondego at freshwater sector and Polychaeta were also more abundant at oligohaline and mesohaline areas. On the contrary, at Mira, Total meiofauna and Nematoda densities were higher at polyhaline area, while Polychaeta and Copepoda exhibited higher densities at the euhaline area.

In both estuaries, the spatial patterns regarding meiofauna abundance and taxa composition clearly reflected salinity gradients. In fact, densities increased from freshwater to seaward areas, and three distinct assemblages were identified: (i) Freshwater areas, where Total meiofauna, Nematoda and Harpacticoids copepods presented the lowest densities in the whole estuary; (ii) Oligohaline and Mesohaline

areas, which presented low Total meiobenthic densities and low diversity; (iii) Polyhaline and Euhaline areas, where Meiofauna reached the highest densities and diversity. Additionally, other environmental factors, such as granulometry, nutrients concentration and sediment organic matter content, also played an important role in structuring meiofauna communities, overlapping and masking salinity effects, namely in the Euhaline areas of both estuaries. In general, the salinity gradient appears strongly linked to sediments properties, originating "site structures" consisting of a complex set of environmental and biotic factors, interacting and regulating meiofauna communities.

Keywords: subtidal meiobenthos, salinity gradient, community patterns, estuaries, macro-scale, meso-scale.

INTRODUCTION

Over the last two decades meiofaunal research has been spread to embark studies of density and distribution and to relate them with environmental factors in coastal lagoons and estuaries (e.g. Coull, 1988; Castel et al., 1990; Castel, 1992; Beier & Traunspurger, 2003; Nozais et al., 2005). The meiofauna of estuarine and marine sediments has, typically, strongly heterogeneous distribution and horizontal patchiness is particularly evident due to the variation of abiotic conditions along estuaries, such as salinity fluctuation (Atrill, 2002), tide action (Smol et al., 1994) and variation of temperature and sediment granulometry (Coull, 1999). Moreover, the spatial distribution of estuarine meiofauna is also influenced by biotic parameters such as food availability (Lee et al., 1977; Soetaert & Herman, 1995), competition (Coull & Venberg, 1975) and the activity of large macrofauna (Coull, 1988; Hall et al., 1994).

Although investigation on the distribution of meiobenthos at different spatial scales is vast (Mokievsky et al., 2004), the knowledge of the composition and abundance of meiobenthos in subtidal estuarine ecosystems is still very scarce. Besides, among European estuaries, there is a notorious unbalance between the northern systems, relatively well studied, and the southern ones, which are poorly known. It is well recognized that the salinity regime in estuaries is a key factor that controls the meiofauna communities (Udalov et al., 2005) with regard to species composition, abundance and diversity (Soetaert et al., 1995). Nevertheless, knowledge regarding the influence of the salinity gradient on the quantitative and qualitative parameters of meiobenthos populations is rather fragmentary (Udalov et al., 2005). For instance, with regard to intertidal meiobenthic communities, a number of studies have been performed within narrow ranges of salinity (e.g. Capstick, 1959; Warwick & Gee, 1984; Austen & Warwick, 1989; Soetaert et al., 1995), but only few cover a wide range

of salinity regimes (Smol et al., 1994). Since other factors, such as grain size and organic matter content in sediments, also correspond to estuarine gradients, the precise influence of salinity on meiofauna is difficult to discern (Udalov et al., 2005). As a consequence, there are contradictory indications of both a significant increase in density of meiobenthos in desalinated waters (Gerlach, 1971) and of a tendency to a decrease in abundance and number of species as one move from the sea to freshwater (Austen & Warwick, 1989; Soetaert et al., 1995).

This paper aims at contributing to fill the gap of knowledge regarding the quantitative distribution of meiofauna communities in Southern European estuaries through 1) a comparative study of the composition and density of meiofauna in subtidal sediments along salinity gradients in two Portuguese estuaries, and by (2) analysing the main environmental variables influencing the structure and distribution of meiofauna assemblages. Two main questions were addressed: a) How does the composition and density of meiofauna communities in subtidal sediments vary along the salinity gradient? and b) Do the distinct anthropogenic pressures observed in the two estuaries cause significant differences in terms of composition and density of meiofauna communities?

MATERIAL AND METHODS

Study areas

This comparative study was carried out simultaneously at the Mondego and Mira estuaries, both located in the Western Coast of Portugal (Fig.1).

The Mondego estuary (40° 08'N, 8° 50'W) is a 21 km long warm-temperate intertidal system (Fig. 1A). Its terminal part consists of two arms, northern and southern, separated by an alluvium-formed island (Murraceira Island), which joined again near the mouth. The two arms have very different hydrological characteristics. The southern arm is shallower (2-4 m during high tide, tidal range 1-3m), presenting large areas of intertidal mudflats (almost 75% of the area) exposed during low tide (Neto et al., 2008). The northern arm is deeper (5-10 m during high tide, tidal range 0.5-3.5m) and receives most of the system's freshwater input, being therefore strongly influenced by seasonal fluctuations in water flow (Flindt et al., 1997). In general, the Mondego estuary is under severe environmental stress, supporting several industries and receiving the agricultural run-off from rice and corn fields in the Lower River valley (Lillebo et al., 2007). Moreover, the Figueira da Foz harbour is located in the northern arm, where regular dredging is carried out to ensure shipping conditions. In the south arm, clear eutrophication symptoms have been observed since the early 1990s, namely the occurrence of seasonal blooms of *Ulva* spp. and a concomitant severe

reduction of the area occupied by *Z. noltii* beds, previously the richest habitat in terms of productivity and biodiversity (Marques et al., 1993), and also of *Z. noltii* biomass in the areas where it still remained (Marques et al., 1997), as a function of the competition with macroalgae (Marques et al., 2003). As a consequence, several interventions were gradually undertaken since 1997 to decrease eutrophication symptoms and to test ways of ameliorating the system's condition (Lillebø et al., 2005; 2007; Neto et al., 2008).

The Mira estuary (37°40'N, 8°40'W) (Fig.1B) constitutes a narrow estuary, nearly 30 km long, with a mean depth of approximately 6 m and bordered by 285 ha of salt-marshes. Together with its surrounding area is included in a protected area, the Natural Park of "Sudoeste Alentejano e Costa Vicentina". This estuary is considered relatively undisturbed and free from industrial pollution (Costa et al., 2001), and the physical and chemical fluctuations result mainly from: a) its morphology, since the terminal section of the river is rather regular, which facilitates the upstream tidal penetration, and b) the concentration of rainfall between January and March, while the rest of the year is usually dry in the region, which determines a normally reduced outflow (Andrade, 1986). Consequently, the estuary is negative in several periods of the year.

Sampling

Sampling was carried out in the Summer of 2006 at both estuaries. Sampling stations were previously allocated to each of the five Venice salinity classes (Anon, 1959) (freshwater < 0.5; oligohaline 0.5–5; mesohaline 5–18; polyhaline 18–30 and euhaline >30) (Table 1). Nine sampling stations were utilised in the Mira estuary (stations 1 to 9) and 11 stations in the Mondego estuary (stations 10 to 20), covering the northern arm (stations 15 and 16) and southern arm (stations 17 to 20) subsystems.

At each station, samples of subtidal meiobenthos consisted of three replicates collected by forcing a "Kajak" sediment corer, with 4.6 cm of inner diameter, 3 cm into the sediment. All samples were preserved in 4% buffered formaldehyde. Meiofauna was afterwards extracted from the sediment fraction using "Ludox HS-40" colloidal silica at a specific gravity of 1.18g cm⁻³ and using a 38 µm sieve (Vincx, 1996). All meiobenthic organisms were counted and identified at higher taxonomic level under a stereomicroscope. Meiofauna taxa identification was based on Higgins & Thiel (1988) and Giere (1993).

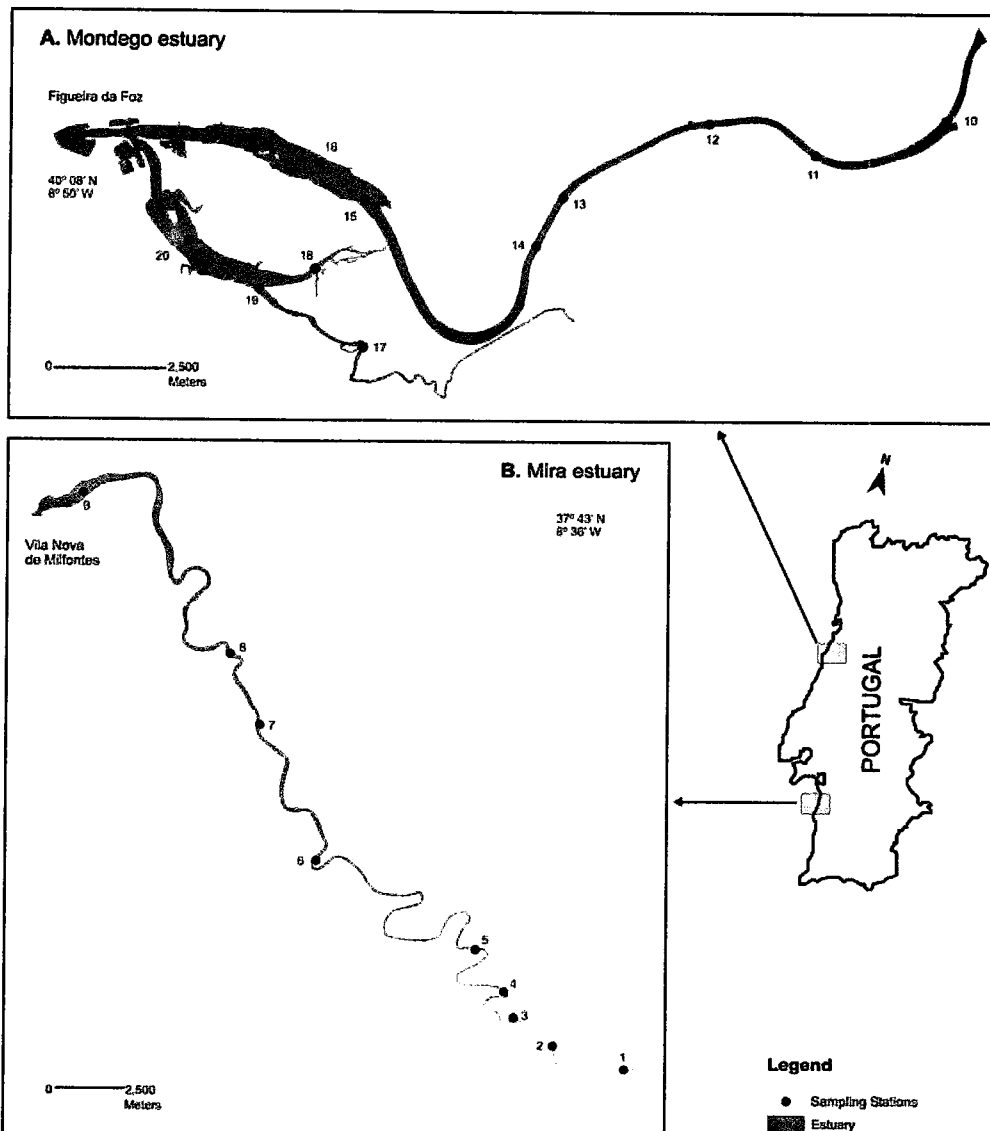


Figure 1 – Station location (black circles) in A. Mondego estuary and B. Mira estuary.

Table 1. Salinity classes from the Venice salinity classification and correspondence with the sampled stations in the Mira and Mondego estuaries.

Venice classification	Salinity ranges	Mira stations	Mondego stations
Freshwater	<0.5	1	10
Oligohaline	0.5-5	2,3,4	11,12
Mesohaline	5-18	5,6,	13,14
Polyhaline	18-30	7,8	17,18,19
Euhaline	>30	9	15,16,20

Along with meiofauna sampling, water salinity, temperature, pH, dissolved oxygen (DO) and transparency were measured *in situ*. $\text{PO}_4^{3-}\text{-P}$, $\text{NO}_3^{-}\text{-N}$, $\text{NO}_2^{-}\text{-N}$ and $\text{NH}_4^{+}\text{-N}$ were determined in the laboratory from water samples, using standardized methods. Additional samples of sediment were collected at each station to analyse the

organic matter content (*OM*) and particles size. Sediment grain size was classified in five classes in accordance to Brown & McLachland (1990): gravel (>2mm), coarse sand (0.500-2.000mm), mean sand (0.250-0.500 mm), fine sand (0.063-0.250 mm) and silt + clay (< 0.063 mm), and the different fractions expressed as percentage of the total sample weight.

Data analysis

Data were analysed in order to a) characterise the distribution of meiobenthic communities along the salinity gradient in both estuaries, taking into account their composition and density; b) find possible differences between estuaries; and c) relate meiofauna assemblages with environmental factors.

Differences observed among stations located at the same salinity stretches in each estuary were compared by means of one-way ANOVA and Mann-Whitney test, using the Statistica 6.0 and GMAV5 for Windows (Underwood & Chapman, 1997) soft wares.

Moreover, multivariate analysis was applied according to the procedures described by Clarke (1993), using the software PRIMER (Plymouth Marine Laboratory, UK). Physicochemical data were in first place normalized and submitted to square root transformation, with the exception of dissolved oxygen and pH, and then underwent Principal Component Analysis (PCA). Data on Meiofauna density suffered square root transformation and then underwent a non-metric multidimensional scaling (nMDS) analysis using the Bray-Curtis similarity index. The contribution of each taxa for the dissimilarities between the groups of stations, previously defined by the multivariate analyses, was determined by using the similarity percentage analysis procedure (SIMPER) and the ANOSIM test was used to analyse differences between stations (Clarke, 1993). The relationship between the environmental variables and the meiobenthic community structure was explored by carrying out BIOENV analysis (Clarke & Ainsworth, 1993).

RESULTS

Abiotic factors

Salinity gradients were observed in both systems. In the Mira estuary, salinity increased from station 1 to station 9 (Fig. 2A). In the Mondego estuary, salinity also increased from station 10 to the station 16, in the downstream section of the northern arm, and in the southern arm it increased from station 17 to station 20 (Fig. 2B). Values of the different environmental factors measured along these gradients are provided in table 2.

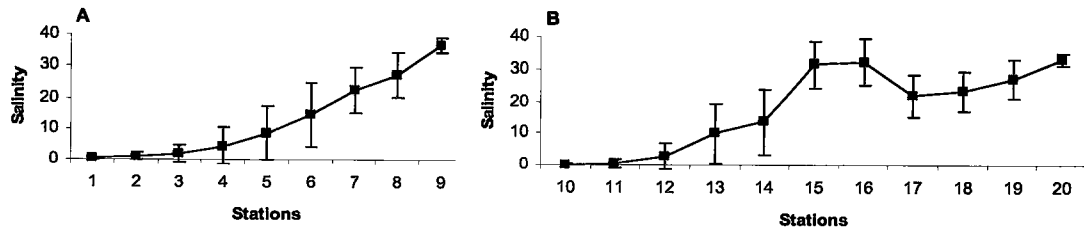


Figure 2 – Mean salinity values and standard deviation at sampling stations in A- Mira estuary and B- Mondego estuary.

In the Mira, the proportion of fine particles in the sediments increased from the upstream sections towards the mouth of the estuary. The three uppermost stations, as well as station 5, exhibited a predominance of coarse sediments (gravel + coarse sand > 64%). The other sampling stations were characterized by sediments with a predominance of particles less than 0.250 mm in diameter (fine sand + silt + clay > 86%), with the exception of the station 9, which presented a sandy bottom (sand > 90%). Typically, sediments characterised by a predominance of fine particles also presented highest percentages of organic matter content (OM). In the Mondego estuary upstream section, bottoms consisted predominantly of fine sand, with the exception of station 10, where the proportion of gravel + coarse sand was approximately 82%, exhibiting also the lowest OM content in the sediments. On the other hand, stations located along the northern arm presented coarse sediments bottoms (more than 50% gravel and coarse sand), while in the southern arm bottoms consisted mainly of mean and fine sand). Fine sand bottoms in the estuarine upper section and in the southern arm presented higher OM contents in sediments, with the highest value occurring at station 17 (6.8%).

In the Mira, water nitrites and ammonium presented the highest concentrations in station 5 (0.019 and 0.126 mg L⁻¹, respectively), with values clearly decreasing towards both the mouth and uppermost section of the estuary. In the Mondego the concentrations of nitrates (minimum 0.00 mg/l, maximum 0.77 mg/l) and phosphates (minimum 0.004 mg/l, maximum 0.016 mg/l) in the water column showed some spatial heterogeneity, but in general the nutrients concentration (PO₄³⁻-P, NO₃⁻-N, NO₂⁻-N and NH₄⁺-N) was higher in the upstream section, decreasing towards the mouth of the estuary.

No significant variations in pH values were detected along the Mira estuarine gradient (the mean value was 7.6), while in the Mondego pH was higher in the southern arm (mean value of 7.9) than in the northern arm (mean value of 7.5), although the average value was similar to the Mira (7.6).

The average temperature was $24.2 \text{ }^{\circ}\text{C} \pm 0.9 \text{ }^{\circ}\text{C}$ in the Mira, with a minimum of $19.5 \text{ }^{\circ}\text{C}$ and a maximum of $27.2 \text{ }^{\circ}\text{C}$, while in the Mondego the average values recorded were lower ($21.7 \pm 0.7 \text{ }^{\circ}\text{C}$), with a maximum of $24 \text{ }^{\circ}\text{C}$ at station 10, in the upstream section. Dissolved oxygen (DO) concentrations in the Mira reached maximum values at stations 3 and 7 (5 mg/l), with a minimum being recorded at station 1 (3.6 mg/l), the innermost one. In the Mondego, DO increased from the inner sections to the mouth of the estuary, and the average concentration ($7.3 \pm 0.3 \text{ mg/l}$) was higher than in the Mira. As for water transparency, the highest values in the Mira were observed at station 9 (1.5m) and the minimum at stations 1 and 2 (0.3m). In the Mondego, transparency also increased from the upstream section towards the mouth along the northern arm, but stations in the southern arm presented both the minimum (0.5 m) and maximum (3.2 m) values recorded.

Regarding the Mira estuary, PCA analysis of physicochemical environmental factors allowed a clear distinction of three groups of stations (Fig 3A): *Group I*, included oligohaline, mesohaline and polyhaline stations, *Group II*, included freshwater, oligohaline and mesohaline stations, and *Group III*, included only station 9, which is euhaline. Station 8 was excluded from the analysis due to lack of data on nutrients. The first two principal components (PC1 and PC2) explained 73.7% of the total variability of the environmental variables in this estuary. Variability along PC1 was mainly explained by the opposition between stations presenting an increase in the bottom sediments of mean sand, and decreasing concentration of nitrates, nitrites, ammonium and total organic carbon concentrations. Variability along PC2 was mainly explained by the opposition between stations presenting higher salinity values and higher proportions of silt + clay in the sediments, located closer to the mouth, and stations presenting lower salinities and coarser bottom sediments, located upstream in the estuary.

Regarding the Mondego, PCA analysis also allowed identifying three groups of stations based on physicochemical variables (Fig 3B): *Group I*, included polyhaline and euhaline stations (15 to 20) located both in the northern and southern arms, *Group II*, included oligohaline and mesohaline stations, and *Group III* included only station 10, already freshwater. The first two principal components explained in this case 85.6% of the total variability, and therefore a simple 2-D plot provided an excellent summary of the whole picture. Variability along PC1 was mainly explained by an increase in the concentration of phosphates, nitrates, nitrites and ammonium from the mouth to the inner areas of the estuary, with an opposite trend regarding salinity values. On the other hand, variability along PC2 was mainly explained by the opposition between

stations presenting higher proportions of fine sand + and clay, as well as OM, and stations presenting a higher proportion of gravel and coarse sand in the sediments.

Table 2. Environmental variables measured at each sampling station from the Mira and Mondego estuaries.

Estuary	St.	Transp. (m)	T (°C)	DO (mg/l)	pH	PO ₄ ³⁻ (mg/l)	NO ₃ ⁻ (mg/l)	NO ₂ ⁻ (mg/l)	NH ₄ ⁺ (mg/l)	OM (%)	Gravel (%)	Coarse sand (%)	Mean sand (%)	Fine sand (%)	Silt+ Clay (%)
Mira	1	0.3	19.5	3.6	7.0	0.004	0.054	0.002	0.034	3.4	44.2	19.7	22.3	11.1	2.8
	2	0.3	23.6	4.5	7.4	0.016	0.407	0.004	0.019	4.0	71.3	17.8	5.3	2.1	3.5
	3	0.8	26.0	5.0	7.4	0.010	0.771	0.008	0.023	6.2	42.3	27.4	8.7	6.0	15.7
	4	0.6	26.5	4.5	7.7	0.007	0.477	0.011	0.023	7.2	0.2	5.7	5.9	9.4	78.9
	5	0.7	26.5	4.2	7.5	0.013	0.451	0.019	0.126	5.7	63.0	3.8	2.1	6.0	25.1
	6	0.6	27.2	4.0	7.5	0.008	0.538	0.014	0.064	8.8	1.9	4.9	6.9	18.4	67.9
	7	0.6	24.8	5.0	7.6	0.013	0.195	0.013	0.035	10.5	2.3	0.9	1.4	11.7	83.7
	8	0.8	22.5	4.2	8.0	-	-	-	-	9.9	0.0	1.3	1.5	5.3	91.8
	9	1.5	21.4	4.8	8.1	0.005	0.000	0.001	0.000	2.3	4.8	22.3	39.3	28.7	5.0
Mondego	10	0.6	24.0	6.4	7.4	0.096	1.331	0.060	0.184	0.2	35.8	46.0	16.2	1.9	0.2
	11	0.7	23.6	5.9	7.4	0.093	1.263	0.043	0.130	4.1	8.8	3.1	16.9	64.4	6.7
	12	0.7	23.3	6.2	7.3	0.067	1.134	0.025	0.101	3.0	38.4	1.7	15.9	39.0	5.1
	13	1.1	22.8	6.2	7.3	0.067	1.134	0.025	0.101	3.8	0.2	0.9	14.4	74.1	10.4
	14	1.1	22.8	7.1	7.4	0.054	0.566	0.014	0.092	4.8	1.1	11.4	16.2	59.1	12.2
	15	2.8	19.0	7.6	7.5	0.045	0.372	0.010	0.066	1.4	29.7	26.3	22.0	17.5	4.5
	16	3.1	18.3	7.6	7.5	0.045	0.372	0.010	0.066	2.5	25.7	26.7	17.7	22.5	7.4
	17	0.5	23.4	8.6	7.8	0.031	0.088	0.004	0.046	6.8	0.2	0.9	25.1	68.7	5.1
	18	1.1	22.9	8.4	7.9	0.027	0.146	0.002	0.051	0.6	17.4	37.0	22.9	22.3	0.4
	19	2.0	20.7	7.9	7.9	0.044	0.312	0.007	0.092	1.4	11.8	35.2	23.9	26.6	2.6
	20	3.2	17.6	8.4	7.9	0.030	0.299	0.002	0.042	0.9	1.6	7.9	27.6	60.9	2.0

St., station; Transp, transparency; T, temperature; DO, dissolved oxygen; PO₄³⁻, phosphate; NO₃⁻, nitrate; NO₂⁻, nitrite; NH₄⁺, ammonium; OM, sediment organic matter; gravel, >2mm; coarse sand, 0.5-2.0mm; mean sand, 0.25-0.50mm; fine sand, 0.063-0.250 mm; silt+clay <0.063mm; -, no available data.

PCA analysis of the matrix including both estuaries physicochemical data revealed clear differences between them (Fig. 3C), with the first two principal components explaining 61.3 % of the variability. The variability along PC1 was mainly explained by the estuarine gradient observed in both systems, with an opposition between stations with higher salinity values and higher proportions of fine particles in the bottom sediments, located closer to the mouths, and stations presenting lower salinities and coarser sediments, in the inner areas. Differences between the two systems are nevertheless clear through their separation along PC2. In fact, variability

along PC2 is essentially explained by the opposition between the higher organic matter content and the higher proportions of silt + clay in bottom sediments observed in the Mira (Group I), and higher values of dissolved oxygen and PO_4^{3-} recorded in the Mondego (Group II).

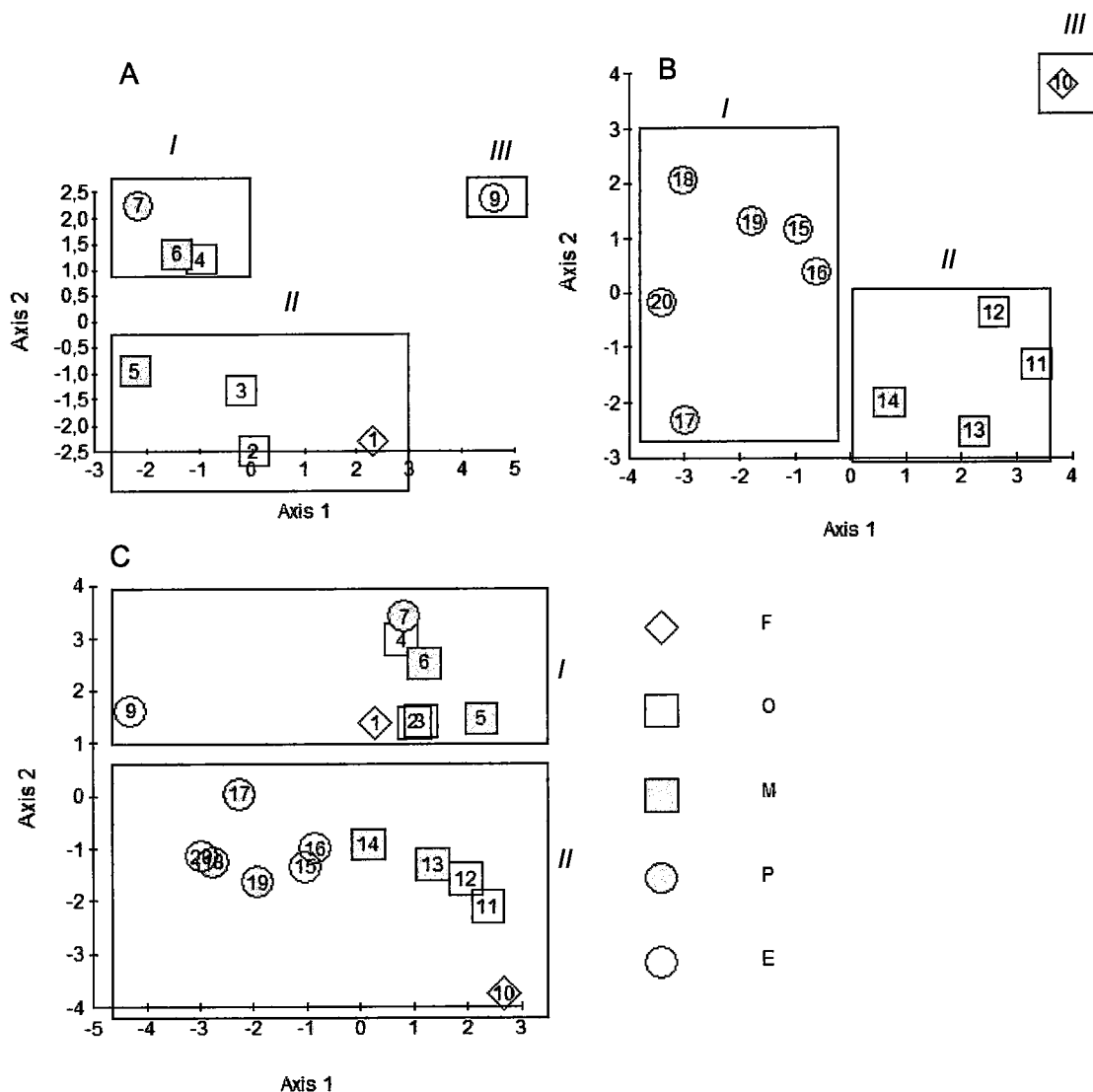


Figure 3 – Principal component analysis (PCA) plot based on the abiotic parameters at each station from A-Mira estuary, (PC1 = 43.2%, PC2 = 30.5%); B- Mondego estuary. (PC1 = 55.2%; PC2 = 30.4%) and C-Mira and Mondego estuaries simultaneously (PC1 = 31.7%; PC2 = 29.6%). F. Freshwater; O. Oligohaline; M. Mesohaline; P. Polyhaline and E. Euhaline.

Meiofauna

Twelve higher taxa were identified from samples carried out at both estuaries: Nematoda, Copepoda, Polychaeta, Ostracoda, Nauplii larvae, Bivalvia, Gastropoda, Halacaroida, Turbellaria, Oligochaeta, Amphipoda, and Cladocera. Ciliophora was only found in the Mondego estuary (Table 3).

The mean meiofauna density was in general higher in the Mira (583.18 ± 159.23 ind. 10cm^{-2}) than in the Mondego (441.65 ± 88.13 ind. 10cm^{-2}). Densities in the Mira ranged from a minimum of 14.45 ± 5.19 ind 10cm^{-2} in station 1 up to a maximum of 2297.41 ± 426.87 ind 10cm^{-2} at station 7, while in the Mondego values ranged from 83.68 ± 20.88 ind. 10cm^{-2} in station 10 to a maximum of 1383.50 ± 397.14 ind. 10cm^{-2} in station 20.

Nematoda was the dominant group in both systems, representing 95% of the total meiofauna in the Mira and 88% in the Mondego. Concerning the other groups, some differences were observed between the two estuaries. Copepoda (2%) were the second most abundant taxa in the Mira, and Polychaeta (1.8%) the third, while all the other taxa represented only approximately 1%. In the Mondego, Polychaeta (8%) were the second most abundant taxa and Copepoda (2%) the third. The remaining taxa represented 2% of the total meiofauna density (Table 3).

With regard to meiofauna composition, differences were also observed between the two arms of the Mondego estuary, since Amphipoda, Turbellaria and Cladocera were absent from the northern arm, and Ciliophora was not found in the southern one. The highest numbers of taxa, 9 and 11, were collected in stations 19 (polyhaline) and 20 (euhaline), respectively.

In the Mira estuary, the maximum density of Nematoda was observed at the polyhaline area (station 7) (2234 ± 400 ind. 10cm^{-2}), while the minimum occurred in freshwater (station 1) (12.44 ± 3.91 ind. 10cm^{-2}) (Fig. 4A). On the other hand, in the Mondego, Nematoda reached the maximum density at the euhaline area in the southern arm (station 20) (1323.10 ± 389.52 ind. 10cm^{-2}), and the minimum, as in the Mira, was recorded in freshwater (station 10) (38.93 ± 5.28 ind. 10cm^{-2}) (Fig. 4B).

Table 3. Mean density \pm SE (number of individuals per 10 cm²) of meiofaunal taxa in each station in the Mira (stations 1 to 9) and Mondego estuary (stations 10 to 20) (-) absence of taxon in the station.

Estuary	St.	Copepoda	Polychaeta	Ostracoda	Nauplii larvae	Bivalvia	Gastropoda	Halacaroida	Turbellaria	Oligochaeta	Amphipoda	Cladocera	Ciliophora
Mira	1	-	0.80 \pm 0.80	-	0.60 \pm 0.35	-	0.20 \pm 0.20	-	-	0.20 \pm 0.20	-	-	0.20 \pm 0.20
	2	1.20 \pm 0.92	6.42 \pm 5.54	1.40 \pm 0.87	0.40 \pm 0.40	5.82 \pm 1.06	-	-	0.20 \pm 0.20	-	-	-	-
	3	1.00 \pm 0.53	3.01 \pm 1.04	2.21 \pm 1.06	1.40 \pm 1.12	0.20 \pm 0.20	-	0.18 \pm 0.20	2.01 \pm 1.45	0.20 \pm 0.20	0.20 \pm 0.20	-	-
	4	1.81 \pm 1.52	0.80 \pm 0.80	8.23 \pm 2.01	1.20 \pm 0.92	-	-	1.61 \pm 0.53	0.60 \pm 0.60	-	-	-	-
	5	0.80 \pm 0.80	0.60 \pm 0.60	1.40 \pm 0.40	-	-	-	-	0.20 \pm 0.20	-	-	-	-
	6	5.22 \pm 1.64	28.50 \pm 7.49	3.01 \pm 2.71	0.40 \pm 0.40	-	0.20 \pm 0.20	1.00 \pm 0.60	0.20 \pm 0.20	-	-	0.20 \pm 0.20	-
	7	54.18 \pm 23.41	3.41 \pm 1.61	2.21 \pm 1.64	0.80 \pm 0.40	0.40 \pm 0.40	0.20 \pm 0.20	1.61 \pm 0.72	0.20 \pm 0.20	0.20 \pm 0.20	0.20 \pm 0.20	0.20 \pm 0.20	-
	8	9.03 \pm 2.17	6.22 \pm 4.54	1.00 \pm 1.00	-	-	4.62 \pm 4.62	0.40 \pm 0.20	0.20 \pm 0.20	1.00 \pm 1.00	-	-	-
	9	36.12 \pm 20.23	44.75 \pm 13.47	0.20 \pm 0.20	4.82 \pm 2.62	-	-	-	-	1.40 \pm 1.40	0.60 \pm 0.35	-	-
Mondego	10	3.01 \pm 1.39	37.53 \pm 15.93	0.20 \pm 0.20	0.40 \pm 0.20	3.01 \pm 0.35	-	-	-	-	0.60 \pm 0.35	-	-
	11	1.00 \pm 0.20	34.12 \pm 9.21	-	0.20 \pm 0.20	33.92 \pm 31.25	-	-	-	-	-	-	-
	12	0.60 \pm 0.35	15.85 \pm 4.12	-	-	-	-	-	-	1.40 \pm 0.20	-	-	-
	13	0.40 \pm 0.20	46.56 \pm 10.81	1.00 \pm 0.20	-	0.20 \pm 0.20	-	-	-	1.00 \pm 1.00	0.20 \pm 0.20	0.20 \pm 0.20	0.60 \pm 0.60
	14	4.01 \pm 1.57	81.08 \pm 35.73	1.40 \pm 1.12	-	0.80 \pm 0.53	-	0.20 \pm 0.20	0.40 \pm 0.20	-	0.20 \pm 0.20	-	-
	15	6.82 \pm 4.12	24.08 \pm 5.14	-	0.60 \pm 0.35	0.80 \pm 0.53	2.01 \pm 1.12	-	-	1.20 \pm 0.60	-	-	3.61 \pm 3.03
	16	1.20 \pm 0.60	9.43 \pm 2.65	0.20 \pm 0.20	0.20 \pm 0.20	1.61 \pm 0.87	-	0.20 \pm 0.20	-	5.22 \pm 3.34	-	-	-
	17	5.42 \pm 1.94	57.80 \pm 10.89	2.21 \pm 1.06	-	0.20 \pm 0.20	-	1.61 \pm 0.20	-	-	-	-	-
	18	35.32 \pm 31.45	52.98 \pm 12.95	15.45 \pm 4.52	0.20 \pm 0.20	1.20 \pm 0.70	-	1.81 \pm 0.92	-	1.00 \pm 0.20	-	-	-
19	2.01 \pm 1.00	16.05 \pm 4.59	-	0.20 \pm 0.20	0.40 \pm 0.20	0.20 \pm 0.20	0.20 \pm 0.20	-	0.20 \pm 0.20	0.40 \pm 0.20	0.40 \pm 0.20	-	
20	30.91 \pm 8.08	4.82 \pm 1.25	4.01 \pm 1.91	5.22 \pm 2.37	6.42 \pm 0.40	3.21 \pm 1.78	-	0.60 \pm 0.35	4.01 \pm 0.87	0.80 \pm 0.20	0.40 \pm 0.40	-	

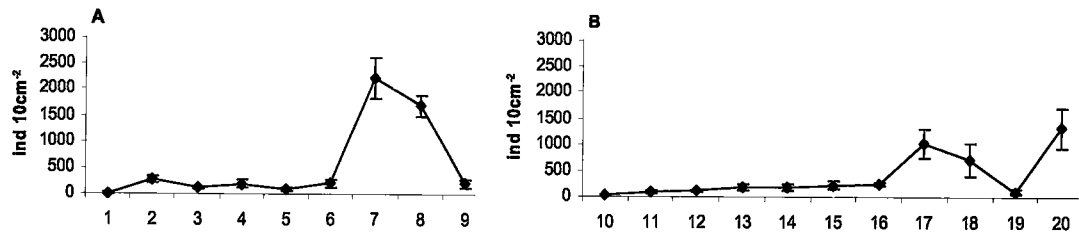


Figure 4 – Mean density \pm SE of Nematoda (ind. 10 cm⁻²) at sampling stations in A- Mira estuary and B- Mondego estuary.

With regard to the total meiofauna density, and to the densities of the most abundant taxa, taking into account stations within a similar salinity range, differences between the two estuaries were analysed using one-way ANOVA and the Mann-Whitney test. In the freshwater section, Nematoda, Copepoda, Polychaeta and total meiofauna densities were significantly higher in the Mondego estuary ($U=0.00$; $p<0.05$ in all). Polychaeta also presented a significantly higher density at the oligohaline ($F=6.9$, $p<0.01$) and mesohaline ($F=7.1$, $p<0.05$) sections in the Mondego estuary. In the polyhaline section, both Nematoda ($F=9.4$, $p<0.001$) and Total meiofauna ($F=8.5$, $p<0.01$) presented significantly higher densities in the Mira estuary, although Polychaeta were significantly more abundant in the Mondego ($F=10.3$, $p<0.01$). Finally, regarding the euhaline zone, the two systems presented significant differences in all the analysed groups, with Nematoda ($F=9.2$, $p<0.01$) and Total meiofauna ($F=8.6$, $p<0.01$) reaching higher densities in the Mondego estuary while Copepoda ($F=4.5$, $p<0.05$) and Polychaeta ($F=10.1$, $p<0.01$) presented significantly higher densities in the Mira estuary.

The MDS analysis of meiofauna data, using the three replicates collected at each station in both systems, indicated that replication was reasonably good (Mira estuary: stress=0.07; Mondego estuary: stress=0.1). Therefore, for simplicity of representation, further analysis was carried out with combined replicates.

The MDS plot based on data from the Mira clearly reflected the meiofauna distribution in stations along the estuarine gradient (stress = 0.01) (Fig. 5A), with densities increasing from the freshwater to the polyhaline section. This MDS plot allowed recognizing three distinct groups: *Group I*, which includes only station 1, in the freshwater section; *Group II*, which includes stations 2 to 6, in the inner parts of the estuary, and station 9, in the euhaline section; and *Group III*, which includes stations 7 and 8, in the polyhaline section. The SIMPER analysis (Table 4) showed higher dissimilarities between groups *I* and *III* (85.8%). The mean density of Nematoda and Copepoda increased from Group *I* to Group *III*, and Polychaeta density was higher in

stations from Group II. Three taxa (Nematoda, Copepoda and Polychaeta) accounted for most of the dissimilarity between the three groups. The low density of Nematoda in the upstream section had a contribution of 79.4% for the dissimilarity between groups I and III, while Copepoda and Polychaeta, which presented higher densities in group III, accounted for 9.2% and 3.1%, respectively, of the dissimilarities between these groups. One of the ANOSIM test requirements is having samplings consisting of four replicates. Although this condition was not fulfilled in the present study (see above), the ANOSIM test was applied taking only into consideration the R value (and not to the significance value) and showed that the composition of meiofauna differed significantly among stations ($R=0.71$).

The BIOENV analysis showed that a combination of four variables (% silt + clay, oxygen concentration, pH and nitrate concentration) explained 50% of the variability found in the meiofauna community (Spearman rank correlation coefficient $r = 0.50$). On the other hand, although results from BIOENV did not detect correlation with salinity, Copepoda and total meiofauna densities showed a significant correlation with this factor ($R = 0.83$, $p < 0.05$; $R = 0.72$, $p < 0.05$, respectively).

The MDS analysis of data on the Mondego estuary communities (Fig. 5B) essentially reflected the distribution of meiofauna in stations along the system, with the exception of station 19, and allowed the recognition of 4 groups: Group I, including station 20, which presented the highest salinity, Group II, which includes stations 17 and 18, located in the southern arm, Group III, including stations 15 and 16, in the northern arm, and 13 and 14, already located in the upstream section, and Group IV, which includes stations 10 to 12, in the freshwater and oligohaline sections, and station 19, located in the polyhaline section of the southern arm. The ANOSIM test allowed the recognition of significant differences between sampling stations ($R=0.61$). Moreover, SIMPER analysis showed a maximum dissimilarity between groups I and IV (60.2%), which was mostly due the highest density of Nematoda in Group I and the lowest in Group IV (Table 4). Finally, the BIOENV analysis, showed that the highest rank correlations occurred between meiofauna and ammonium concentration in the water ($r=0.682$), and between meiofauna and the concentrations of ammonium and phosphates ($r=0.666$). Nematoda ($R=0.75$, $p < 0.05$), Gastropoda ($R=0.67$, $p < 0.05$), and Oligochaeta ($R=0.65$, $p < 0.05$) showed a positive correlation with salinity.

Differences between the two systems based on the comparison of their meiofauna assemblages (Fig.5C) were not as evident as the ones resulting from their physicochemical characterization (Fig. 3C). Actually, the analysis of the matrix including data on meiofauna from the two systems did not separate the stations from both estuaries. Instead, the MDS plot allowed recognizing three distinct groups: Group

I, which includes stations from the southern arm of the Mondego estuary and also from the terminal area of the Mira estuary, all located in the polyhaline and euhaline sections of the estuaries; Group II, which includes stations from all the salinity classes; and Group III, which includes only a station in the freshwater section of the Mira estuary. Nematoda, Polychaeta and Copepoda exhibited clear increasing densities from stations belonging to Group III to stations from Group I, with these groups presenting a dissimilarity of 84.0%. Differences between Group II and Group III (dissimilarity of 67.0%) were higher than between Group II and Group I (dissimilarity of 51.5%) (Table 4). The SIMPER analysis applied to the matrix including data on both systems presented Nematoda, Copepoda and Polychaeta, the three most abundant taxa, as responsible for the three groups formed.

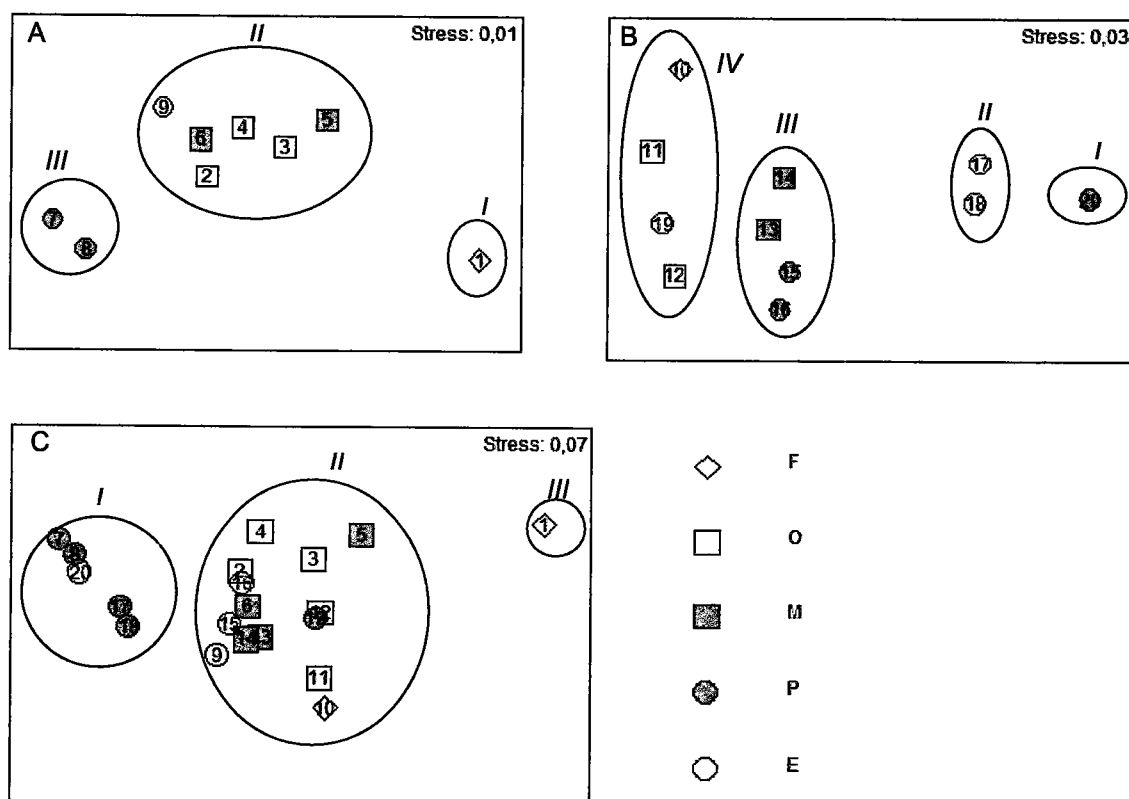


Figure 5 – Non-metric multidimensional scaling (nMDS) plot based on the density of meiofaunal taxa from sampling stations A- Mira estuary, B- Mondego estuary and C- Mira and Mondego estuaries simultaneously. F. Freshwater; O. Oligohaline; M. Mesohaline; P. Polyhaline and E. Euhaline

Table 4. Taxa determined by SIMPER as those most responsible for contributing for the similarity within each group (group determined by MDS) for the Mira estuary, the Mondego estuary and the Mira and Mondego estuary simultaneously. Shaded boxes: percentage of similarity (bold) and taxa that contributed for similarity between stations in each group. Non-shaded box, percentage of dissimilarity (bold) between two groups and percentage of dissimilarity that each taxon contributed for the total dissimilarity. N. Nematoda; C. Copepoda; P. Polychaeta; O. Oligochaeta and B. Bivalvia.

Mira estuary	Group I	Group II	Group III	-
	58.9%			-
Group I	N:93.1%			-
				-
	66.5%	64.1%		-
Group II	N: 52.3%	N:77.7%		-
	P:13.0%	P:7.8%		-
	C: 8.8%	O: 6.0%		-
	85.8%	56.9%	83.7%	-
Group III	N:79.4%	N:74.2%	N:88.5%	-
	C:9.2%	C:8.4%	C: 7.2%	-
	P:3.1%			-
Mondego estuary	Group I	Group II	Group III	Group IV
	79.9%			
Group I	N:65.3%			
	C:10.3%			
	B: 5.6%			
	31.8%	79.2%		
Group II	N: 34.6%	N:67.5%		
	P: 16.9%	P: 18.9%		
	C: 11.1%	C: 5.0%		
	48.8%	36.3%	73.4%	
Group III	N: 53.4%	N: 55.4%	N: 67.6%	
	C: 10.2%	P:11.3%	P: 23.3%	
	60.2%	49.6%	32.0%	71.7%
Group IV	N: 59.2%	N: 62.2%	N: 41.4%	N: 58.8%
	C: 9.8%	P: 9.4%	P: 17.5%	P: 31.2%
Mira & Mondego	Group I	Group II	Group III	-
	74.3%			-
Group I	N: 79.9%			-
	C: 7.4%			-
	P: 6.8%			-
	51.5%	65.6%		-
Group II	N:64.4%	N: 70.9%		-
	P: 9.1%	P:18.5%		-
	C: 8.5%	C: 5.2%		-
	84.0%	67.0%	58.92	-
Group III	N: 68.7%	N: 47.9%	N: 93.1%	-
	C: 8.9%	P: 21.2%		-
	P: 8.8%	C: 7.8%		-

DISCUSSION AND CONCLUSIONS

Gradients of salinity and sediments particle size were clearly detected at both estuaries.

In the Mira, the observed salinity gradient is mostly explained by the morphology of the estuary - a single river channel and an almost complete absence of irregularities

in its terminal section - which allows the tidal influence to extend about 40 km inland (Paula et al. 2006). In the Mondego, due to the distinct hydrological regimes of the northern and southern arms, two salinity gradients are recognizable. The northern arm is deeper and has been heavily modified, namely in the last two decades, by the construction of stonewalls along the river banks and of small water reservoirs for aquaculture, which caused changes in hydrodynamics and had a strong anthropogenic impact. The tidal penetration is therefore faster along the northern arm and salinity is higher than in the southern arm during high-water periods. On the contrary, the south arm is much shallower, almost constituting a kind of coastal lagoon in which the water circulation depends mostly on the tides and on the freshwater input on the irregular discharge, controlled by a sluice, from a small tributary, the Pranto River (Cardoso et al., 2004).

A typical gradient of estuarine sediments was observed in the Mira, with fractions of silt and clay increasing from upstream sections to the mouth of the estuary. The only exception was sampling station 9 (sediments consisting of 90% sand), very close to the mouth, due to its location on top of a sand flat. On the contrary, the gradient of estuarine sediments was much less clear in the Mondego estuary. In general, in the Mondego estuary, subtidal bottoms presented larger fine sand fractions and much smaller silt + clay fractions than in the Mira. Moreover, sediments distribution pattern was found to be unlike in the two arms (Teixeira et al., 2007). Along the northern arm particles size increased from the mouth to upstream sections, where bottoms consisted mainly of coarse sand. On the contrary, in the southern arm particles size decreased from the mouth to the inner most sections.

In both estuaries, the organic matter content in sediments increased as the particle size decreased, which is obviously related to the fact that fine sediments have a higher surface area for organic adsorption (Dale *in* Parsons, 1990). In the Mira, nutrients concentrations did not show any spatial pattern of variation during the sampling period, remaining constant along the estuary, which may be explained by the absence of significant inputs related with anthropogenic activities. On the contrary, in the Mondego, nutrients concentrations [ammonium (NH_2), the oxidised forms of nitrogen, and phosphates] were higher in the northern arm than in the southern one, decreasing seawards in both arms. Actually, a previous study in the Mondego estuary suggested a strong dependency of the concentration of oxidised forms of dissolved nitrogen on the freshwater inputs from diffuse and/or point sources, which may include precipitation and the consequent freshwater flow with agricultural lands draining, as main sources of nitrate (Lillebo et al., 2007).



Meiobenthos density and composition in subtidal sediments showed a pattern of distribution which was clearly related with the salinity gradient both in the Mondego and Mira estuaries, but the observed variations were also related to other physicochemical factors. In both estuaries, all along the salinity gradient, meiofauna assemblages were strongly dominated by Nematoda (93% in the Mira and 47 to 96% in the Mondego), which is in agreement with other studies. In fact, meiofauna of many lakes, rivers, marine and estuarine sediments is nearly always dominated by Nematoda (Austen & Warwick, 1989; Soetaert et al., 1995; Udalov et al., 2005). Nevertheless, in the Mondego, the proportion of Nematoda clearly decreased in the freshwater section, as was also observed by Udalov et al. (2005), while in Mira it remained approximately constant. The only exception in the Mira was a sampling station located in the euhaline section, where we observed the lowest proportion (70%). That was related with a higher proportion of sand in the sediments and a concomitant reduction of silt + clay and of the organic matter content. The exception in the Mondego was in a sampling station located in polyhaline section, with low meiofauna density. That was related with the water nutrients input from the agricultural lands draining. Copepoda and Polychaeta came second in relative abundance in the Mira estuary, while in the Mondego the same position belonged to Polychaeta, followed by Harpacticoid copepods. Again, our results agree with previous studies. Actually, Copepoda are typically second in estuarine sediments (Coull, 1999; Mokievsky, 2004), and occasionally, a taxon other than Copepoda is second in order of abundance (Santos et al., 1996).

Total meiobenthos density varied from 15 ind. 10 cm^{-2} (freshwater section) up to 2297 ind. 10 cm^{-2} (polyhaline section) in the Mira estuary, and from 84 ind. 10 cm^{-2} (freshwater section) up to 1384 ind. 10 cm^{-2} (euhaline section) in the Mondego. Comparable values in subtidal communities and a similar pattern of increasing density seawards have also been reported in subtidal sediments in the Westerschelde estuary (67-1666 ind. 10 cm^{-2}) (Soetaert et al., 1994), and in the intertidal sediments in the Chernaya River (167-2356 ind. 10 cm^{-2}) (Udalov et al., 2005). This illustrates the role of salinity as an important independent factor in the determination of the structure of the meiobenthic communities and in describing the changes of the total meiobenthic density and diversity (Coull, 1985b; Soetaert et al., 1995; Santos et al., 1996; Vincx et al., 1990; Atrill, 2002).

For both estuaries, nMDS ordinations allowed describing community patterns that followed closely the salinity gradient: (i) Freshwater sections (15 to 84 ind. 10 cm^{-2}), where Total meiofauna, Nematoda and Harpacticoid copepods densities presented minimum values, which constitutes a common feature of freshwater communities (Heip et al, 1985; Soetaert et al., 1994; Soetaert et al., 1995; McArthur et al. 2000; Udalov et.

al., 2005); (ii) Oligohaline and Mesohaline sections, where Total meiobenthic densities were low (81 to 292 ind.10cm⁻²) and diversity poor, which is in accordance with several studies (Soetaert et al., 1994; Soetaert et al.,1995; Yamamuro 2000); (iii) Polyhaline and Euhaline sections, where meiofauna reached the highest density values (833 to 2297 ind.10cm⁻²) and the highest diversity. Such pattern has been observed in other estuarine environments and is easily explained. Downstream areas of estuaries are invaded by marine species, which have to adapt to reduced salinities in variable degrees, vanishing with decreasing salinity (Bowmann, 1983). Thus, a) estuarine meiofauna tend to decrease in abundance and number of species as one moves from the sea to freshwater (Austen & Warwick, 1989), and b) since the preponderance of species in estuaries is marine, species richness tend to decrease as one moves towards freshwater (Coull, 1999).

In the present study, although we could confirm the direct effect of salinity on meiofauna communities, we also illustrated that other environmental factors, such as granulometry, nutrients concentration and sediments organic matter content, may interact and prevail over salinity effects. In fact, with regard to meiofauna densities, deviations from the general trend were observed in euhaline part of the Mira estuary, as well as in the northern and southern arms in the Mondego, showing how the highest-effect of the sediments properties may mask salinity effects on meiofauna assemblages.

Therefore, the macro-scale (between estuaries) and mesoscale (within each estuary) approaches undertaken in this study support the notion that there is a close relationship between physicochemical environmental factors and the structure of meiofauna communities inhabiting estuarine sediments. Actually, meiobenthos tends to show a strongly heterogeneous spatial distribution as a function of physical gradients (at the scale of km to dm), which may cause significant variations with regard to meiofauna abundance and community patterns (Soetaert et al., 1994). Consequently, mesoscale variability within estuaries (at the scale of km), due to salinity changes or grain size differences, is often more important than variability at the scale of hundreds of km or between estuaries (Soetaert et al., 1995). On the other hand, micro-scale changes in meiofauna spatial distribution can be related to the aggregation of individuals, e.g. patches, which can be caused by patchy food distribution and by social or reproductive behaviour (Li et al., 1997).

Meiobenthic communities tend therefore to respond very straight to the habitat physicochemical conditions, namely in naturally stressed transitional waters' ecosystems. In terms of management, this represents an obvious constraint to the applicability of ecological quality evaluation tools, e.g. benthic index, to detect changes

of anthropogenic origin. These results come very much along the lines of the “Estuarine Quality Paradox” (Dauvin, 2007), emphasizing the difficulty of distinguishing between natural stress and anthropogenic stress in such highly variable ecosystems as estuaries.

In conclusion, our results confirm, both at macro-scale and meso-scale, that spatial variations of meiofauna densities and composition in estuarine subtidal sediments strongly depend on the salinity gradient, as observed in Northern Europe systems. But show also that salinity was not the specific factor determining the observed community patterns. In fact, the salinity gradient interacted with other sediments properties generating a “site structure” consisting of a complex set of environmental and biotic factors which regulated meiofauna communities.

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Chapter 3

**Spatial distribution of subtidal Nematoda communities along a salinity gradient
in two Southern European estuaries (Portugal)**

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ABSTRACT

The spatial distribution of the nematode community on subtidal sediments in Mira and Mondego estuaries (Portugal), exposed to different degrees of anthropogenic stress, was investigated. Samples were collected at 12 sampling stations, 7 located in Mondego and 5 in Mira estuary, along the salinity gradient and patterns in the density, composition and trophic structure of the community were analysed.

Nematodes densities ranged from 39 to 2234 ind. 10cm⁻² and assemblages composition closely resemble those of the north European estuaries, mainly composed by *Sabatieria*, *Metachromadora*, *Daptonema*, *Anoplostoma*, *Sphaerolaimus* and *Terchellingia*. For both estuaries the patterns of density and composition of the nematode assemblages were alike and followed closely the salinity gradients: *i*) Freshwater and Oligohaline sections, characterised by the presence of freshwater nematodes, low density of the total nematodes and low diversity of genera; *ii*) Mesohaline section, where total nematodes densities and diversity were low and the dominant nematode genera were *Terschellingia*, *Sabatieira*, *Daptonema* and *Anoplostoma*; *iii*) Polyhaline and Euhaline sections, where nematodes reached the highest density and *Paracomesoma*, *Synonchiella*, *Odontophora*, *Sabatieria*, *Metachromadora*, *Daptonema* and *Ptycholaimellus* attained the highest densities. Besides salinity, the most important factors to determine the nematode community structure were sediment grain size, organic matter content and nutrients concentration.

For both estuaries the nematode feeding type composition patterns were remarkably different, with the Mira estuary presenting high dominance of selective deposit feeders in the oligohaline section and the remaining areas being characterised by the dominance of non-selective deposit feeders, while in Mondego estuary, epigrowth-feeders and omnivores/predators were dominant in freshwater and euhaline sections of the southern arm.

Heterogeneity was much higher along the estuarine gradients than between estuaries, indicating that mesoscale variability within estuaries (at the scale of km) was more important than variability at the scale of hundreds of km or between estuaries. Spatial variability was associated to natural stressors characteristics of estuaries, as salinity and sediment gradient and the anthropogenic stressors of the Mondego estuary seemed to be not relevant to nematode community densities patterns. Nevertheless, different patterns of the trophic structure assemblages between Mira and Mondego overlap the salinity effects (natural stressor) and the feeding guilds and their response could detect the anthropogenical-induced stress in estuaries.

Keywords: Nematodes, salinity gradient, subtidal sediments, estuary, feeding-types, Portugal.

INTRODUCTION

Nematode dominance in marine and estuarine meiobenthic communities of the sediments has been largely documented, as well as the importance of salinity and sediment properties on spatial distribution, abundance and species composition of the free-living nematodes community (Austen & Warwick, 1989; Vincx et al., 1990; Coull, 1999).

It is well known that, in estuaries, the salinity is a key independent factor determining the structure of the communities, controlling species composition, abundance and diversity (Soetaert et al., 1995). Nevertheless, knowledge regarding the influence of the salinity gradient on the quantitative parameters of nematodes populations is focused in intertidal sediments, while the subtidal remains less studied. For instance, with regard to intertidal nematode communities, a number of studies have been performed within narrow ranges of salinity (e.g. Austen & Warwick, 1989; Capstick, 1959; Warwick & Gee, 1984) and studies on the spatial distribution of subtidal estuarine nematodes along salinity gradients, from freshwater to euhaline areas are scarce.

The spatial and temporal patterns of estuarine nematode communities have been intensively studied in the intertidal sediments of the eastern coast of the North

Atlantic (e.g. Warwick & Gee, 1984; Heip et al., 1985; Li & Vincx, 1993; Smol et al., 1994; Steyaert, 2003; Rzeznik-Orignac et al., 2003), creating an unbalance among European estuaries, since northern systems are relatively well studied while southern ones remain poorly known (Soetaert et al. 1995, Adão, 2004). The investigations on spatial patterns of temperate nematode communities on different horizontal scales have related them to individual environmental factors, such as sediment grain size (Warwick, 1971, Coull, 1988), organic content (Moens et al., 1999), food resources (Moens & Vincx, 1997) and disturbances of different nature (Warwick & Gee, 1984; Austen & Warwick, 1989).

This paper aims at contributing to fill the gap of knowledge regarding the distribution of subtidal nematodes communities in Southern European estuaries through 1) a comparative study of the densities, genera and feeding groups distributions of nematodes communities along salinity gradients, in two Portuguese estuaries, and by (2) analysing the main environmental variables influencing the structure and distribution of nematodes assemblages in two estuaries exposed to different degrees of anthropogenic stress. Two main questions were addressed: a) How does the composition and density of nematodes community in subtidal sediments vary along the salinity gradient? and b) Are there differences in terms of the nematodes communities features between the two estuaries that are subjected to distinct anthropogenic pressures?

MATERIAL AND METHODS

Study areas

This comparative study was carried out simultaneously at the Mondego and Mira estuaries, both located in the Western Coast of Portugal.

The Mondego estuary (40° 08'N, 8° 50'W) is a 21 Km long warm-temperate intertidal system (Fig. 1A). Its terminal part consists of two arms, northern and southern, separated by an alluvium-formed island (Murraceira Island), which join again near the mouth. The two arms have very different hydrological characteristics. The southern arm is shallower (2-4 m during high tide, tidal range 1-3m), presenting large areas of intertidal mudflats (almost 75% of the area) exposed during low tide. The northern arm is deeper (4-10m during high tide, tidal range 0.5-3.5m) and receives most of the system's freshwater input, being therefore strongly influenced by seasonal fluctuations in water flow (Flindt et al., 1997). In general, the Mondego estuary is under severe environmental stress, supporting several industries and receiving the agricultural run-off from rice and corn fields in the Lower River valley (Lillebø et al., 2007). Moreover, the Figueira da Foz harbour is located in the northern arm, where

regular dredging is carried out to ensure shipping conditions. In the south arm, clear eutrophication symptoms have been observed since the early 1990s, namely the occurrence of seasonal blooms of *Ulva* spp. and a concomitant severe reduction of the area occupied by *Z. noltii* beds, previously the richest habitat in terms of productivity and biodiversity (Marques et al., 1993), and also of *Z. noltii* biomass in the areas where it still remained (Marques et al., 1997), as a function of the competition with macroalgae (Marques et al., 2003). As a consequence, several interventions were gradually undertaken since 1998 to decrease eutrophication symptoms and to test ways of ameliorating the system's condition (Lillebø et al., 2005; 2007; Neto et al., 2008).

The Mira estuary (37°40'N, 8°40'W) (Fig.1B) constitutes a narrow estuary, nearly 30 km long, with a mean depth of approximately 6 m and bordered by 285 ha of salt-marshes. Together with its surrounding area is included in a protected area, the Natural Park of "Sudoeste Alentejano e Costa Vicentina". This estuary is considered relatively undisturbed and free from industrial pollution (Costa et al., 2001), and the physical and chemical fluctuations result mainly from: a) its morphology, since the terminal section of the river is rather regular, which facilitates the upstream tidal penetration, and b) the concentration of rainfall between January and March, while the rest of the year is usually dry in the region, which determines a normally reduced outflow (Andrade, 1986). Consequently, the estuary is negative in several periods of the year.

Sampling strategy

Nematodes were sampled in the Summer of 2006 at both estuaries. Sampling stations were previously allocated to one of the five Venice salinity classes (Anon, 1959) (freshwater < 0.5; oligohaline 0.5–5; mesohaline 5–18; polyhaline 18–30 and euhaline >30) (Table 1). Five sampling stations were analysed in the Mira estuary (stations 2, 3, 6, 7 and 9) and 7 stations in the Mondego estuary (stations 10, 11, 12, 13, 14, 15 and 20), covering the northern (station 15) and southern arm (station 20) subsystems (Fig. 1). At each station, three replicates were collected by forcing a "Kajak" sediment corer, with 4.6 cm of inner diameter, 3 cm into the sediment. All samples were preserved in 4% buffered formaldehyde. Nematodes were afterwards extracted from the sediment fraction using "Ludox HS40" colloidal silica at a specific gravity 1.18 g cm⁻³ and using a 38µm sieve (Heip et al., 1985) and counted under a stereomicroscope. A random set of 120 nematodes, or the total number of individuals (if less than 120 nematodes per sample), were picked from each replicate, cleared in glycerol-ethanol solution, stored in anhydrous glycerol and mounted on glycerine slides

for identification (Vincx, 1996). Genus identification was based on Platt & Warwick (1988) and Warwick et al. (1998).

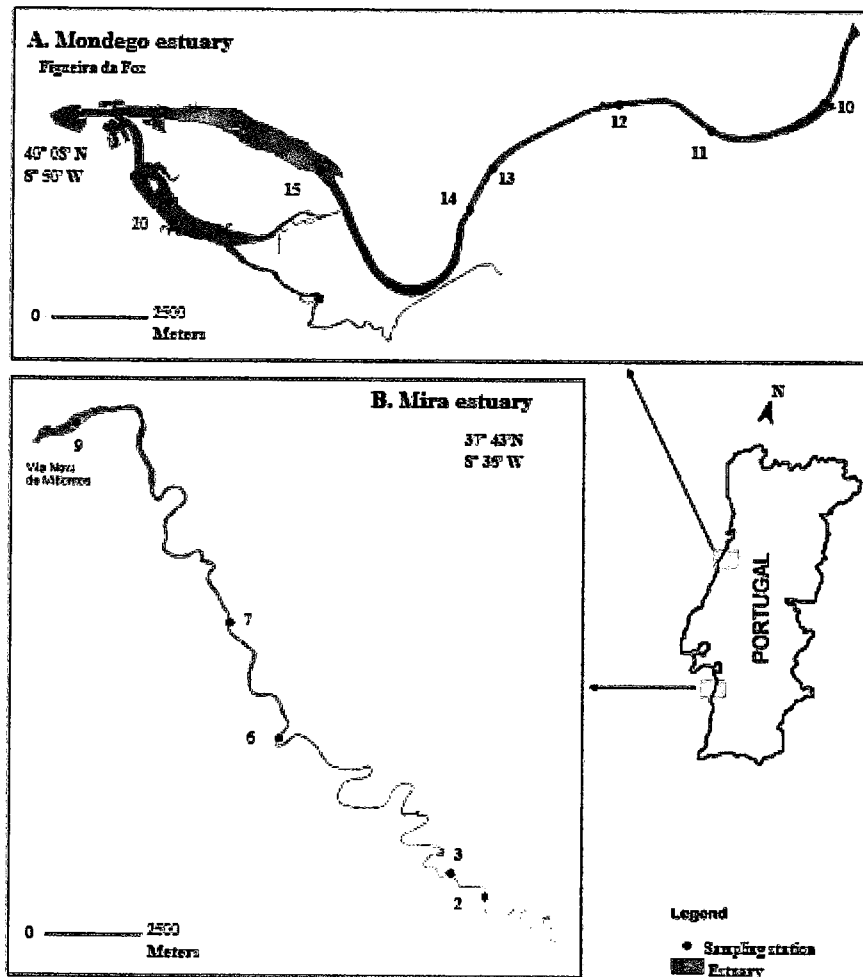


Figure 1 – Station location (black circles) in A. Mondego estuary and B. Mira estuary.

Table 1. Salinity classes from the Venice salinity classification and correspondence with the sampled stations in the Mira and Mondego estuaries.

Venice classification	Salinity ranges	Mira stations	Mondego stations
Freshwater	<0.5	-	10
Oligohaline	0.5-5	2,3	11,12
Mesohaline	5-18	6	13,14
Polyhaline	18-30	7	-
Euhaline	>30	9	15, 20

Along with Nematoda sampling, water salinity, temperature, pH, dissolved oxygen (DO) and transparency were also measured *in situ*. $\text{PO}_4^{3-}\text{-P}$, $\text{NO}_3^{-}\text{-N}$, $\text{NO}_2^{-}\text{-N}$ and $\text{NH}_4^{+}\text{-N}$ were determined in the laboratory from water samples, using standardized

methods. Additional samples of sediment were collected at each station to analyse the organic matter content (*OM*) and particles size. Sediments grain size was classified in five classes in accordance to Brown & McLachland (1990): gravel (>2mm), coarse sand (0.500-2.000mm), mean sand (0.250-0.500 mm), fine sand (0.063-0.250 mm) and silt + clay (< 0.063 mm) and the different fractions were expressed as percentage of the total sample weight.

Data analysis

Data were analysed in order to a) characterise the nematode communities distribution along the salinity gradient in both estuaries, taking in account their composition, density and feeding group, b) find possible differences between estuaries, and c) to relate nematode assemblages with environmental factors.

Total nematode densities within each estuary were compared by means of one-way ANOVA (the square root transformation was applied whenever the assumptions were not met), using the software GMAV5 for Windows (Underwood & Chapman, 1997) and, whenever differences were detected, the *a posteriori* test Student-Newman-Keuls (SNK) was performed.

Multivariate analysis was applied according to the procedures described by Clarke (1993), using the software PRIMER (Plymouth Marine Laboratory, UK). Physicochemical data were in first place normalized and submitted to square root transformation, with the exception of dissolved oxygen and pH, and then underwent Principal Component Analysis (PCA). Data on nematodes density suffered square root transformation and then underwent a non-metric multidimensional scaling (nMDS) analysis using the Bray-Curtis similarity index. The contribution of each genus for the dissimilarities between groups of stations, previously defined by the multivariate analysis, was determined by using the similarity percentage analysis procedure (SIMPER). The relationship between the environmental variables and the nematode community structure was explored by carrying out BIOENV analysis (Clarke & Ainsworth, 1993) and Spearman correlations were performed to identify the existence of correlation between the environmental factors and nematodes that characterised each section of the estuary.

Nematodes were grouped into four feeding guilds, according to the feeding type classification of Wieser (1953), distinguishing selective (1A) and non-selective (1B) deposit-feeders, epigrowth-feeders (2A) and omnivores/predators (2B), to investigate the trophic structure of the community. The percentage of contribution of each feeding group at each replicate from each sampling station underwent a non-metric multidimensional scaling (nMDS) analysis using the Bray-Curtis similarity index and the

contribution of each feeding group for the dissimilarities between groups of stations, previously defined by the multivariate analysis, was determined by using the similarity percentage analysis procedure (SIMPER).

RESULTS

Abiotic factors

At both estuaries, salinity gradients were observed, with an increase in salinity from upstream to downstream. Values of the different environmental factors measured along these gradients are provided in Table 2.

In the Mira, the proportion of fine particles in the sediments increased from the upstream section towards the mouth of the estuary. The upstream section was dominated by coarse sediments while the remaining sections were characterized by sediments with a diameter less than 0.250 mm and the euhaline part was mainly composed by sand. As expected, the finest sediments presented the highest percentage of organic matter content (OM). The upstream section of the Mondego estuary was mostly composed by fine sand, with the exception of the freshwater part, where the proportion of gravel + coarse sand was approximately 82%, exhibiting also the lowest OM content. The North arm presented coarse sediment bottoms, while in the southern arm bottoms consisted mainly of mean and fine sand. Fine sand bottoms in the estuarine upper sections presented higher OM contents in sediments.

In the Mira estuary, water nitrites and ammonium presented the highest concentrations in the mesohaline section, with values clearly decreasing toward both the mouth and uppermost section of the estuary. In the Mondego estuary the concentration of nitrates and phosphates in the water column presented some spatial heterogeneity but, in general, nutrients concentration ($\text{PO}_4^{3-}\text{-P}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NH}_4^+\text{-N}$) was higher in the upstream section, decreasing towards the mouth of the estuary.

No significant variations in the pH values were detected in the Mira along the Mira estuary, while in the Mondego estuary pH was higher in the southern arm than in the northern arm, although the average value was similar to the Mira.

Dissolved oxygen (DO) concentrations in the Mira estuary reached maximum values at oligohaline and polyhaline areas, with a minimum being recorded in the mesohaline section. In the Mondego estuary, DO increased from the head to the mouth of the estuary, and the average concentration was higher than in the Mira. As for water transparency, the highest values in the Mira estuary were observed in the euhaline section and the minimum in the freshwater area. In the Mondego, transparency also

increased from the upstream section towards the mouth along both the northern and southern arm.

Table 2. Environmental variables measured at each sampling station from the Mira and Mondego estuaries.

Estuary	St.	Sal psu	Trp. (m)	T (°C)	O ₂ (mg/l)	pH	PO ₄ ³⁻ (mg/l)	NO ₃ ⁻ (mg/l)	NO ₂ ⁻ (mg/l)	NH ₄ ⁺ (mg/l)	OM (%)	Grv (%)	Coar sand (%)	M. sand (%)	F. sand (%)	Silt+ Clay (%)
Mira	2	1.1	0.3	23.6	4.5	7.4	0.016	0.407	0.004	0.019	4.0	71.3	17.8	5.3	2.1	3.5
	3	2.0	0.8	26.0	5.0	7.4	0.010	0.771	0.008	0.023	6.2	42.3	27.4	8.7	6.0	15.7
	6	14.6	0.6	27.2	4.0	7.5	0.008	0.538	0.014	0.064	8.8	1.9	4.9	6.9	18.4	67.9
	7	22.4	0.6	24.8	5.0	7.6	0.013	0.195	0.013	0.035	10.5	2.3	0.9	1.4	11.7	83.7
	9	36.6	1.5	21.4	4.8	8.1	0.005	0.000	0.001	0.000	2.3	4.8	22.3	39.3	28.7	5.0
Mondego	10	0.1	0.6	24.0	6.4	7.4	0.096	1.331	0.060	0.184	0.2	35.8	46.0	16.2	1.9	0.2
	11	0.5	0.7	23.6	5.9	7.4	0.093	1.263	0.043	0.130	4.1	8.8	3.1	16.9	64.4	6.7
	12	2.7	0.7	23.3	6.2	7.3	0.067	1.134	0.025	0.101	3.0	38.4	1.7	15.9	39.0	5.1
	13	10.0	1.1	22.8	6.2	7.3	0.067	1.134	0.025	0.101	3.8	0.2	0.9	14.4	74.1	10.4
	14	13.6	1.1	22.8	7.1	7.4	0.054	0.566	0.014	0.092	4.8	1.1	11.4	16.2	59.1	12.2
	15	31.6	2.8	19.0	7.6	7.5	0.045	0.372	0.010	0.066	1.4	29.7	26.3	22.0	17.5	4.5
	20	33.1	3.2	17.6	8.4	7.9	0.030	0.299	0.002	0.042	0.9	1.6	7.9	27.6	60.9	2.0

St, station; Sal, salinity; Trp, transparency; T, temperature; O₂, dissolved oxygen; PO₄³⁻, phosphate; NO₃, nitrate; NO₂⁻, nitrite; NH₄⁺, ammonium; OM, sediment organic matter; Grv, gravel (>2mm); Coar sand, coarse sand (0.5-2.0mm); M.sand, mean sand (0.25-0.50mm); F. sand, fine sand (0.063-0.250 mm); Silt+Clay, silt+clay (<0.063mm).

Regarding the Mira estuary, PCA analysis of physicochemical environmental factors allowed a clear distinction of three groups of stations (Fig 2A): Group *I*, included oligohaline stations, Group *II* included mesohaline and polyhaline stations and Group *III*, included the only euhaline station. The first two principal components (PC1 and PC2) explained 85.3% of the total variability of the environmental variables in this estuary. Along PC1, variability was mainly explained by an increase in the proportion of mean sand and an opposite trend in the concentration of nitrates, nitrites and ammonium. Along PC2, variability was mainly explained by the opposition between stations presenting higher salinity values as well as higher proportions of silt+clay in the sediments, located closer to the mouth, and stations presenting lower salinities and coarser bottom sediments, located upstream in the estuary.

Regarding the Mondego estuary, PCA analysis also allowed the identification of 3 groups of stations based on the physicochemical variables (Fig 2B): Group *I*, included only the freshwater station, Group *II*, included oligohaline and mesohaline stations, and Group *III*, included euhaline stations. The first two principal components

explained 87.7% of the total variability. Variability along PC1 was mainly explained by an increase in the concentration of nitrates, nitrites, ammonium and phosphates from the mouth to the inner stations of the estuary, with an opposite trend regarding salinity values. On the other hand, variability along PC2 was mainly explained by the opposition between stations presenting higher proportion of fine sand, silt+clay and OM, and stations presenting higher proportion of gravel and coarse sand in the sediments.

PCA analysis of the joint matrix including both estuaries physicochemical data revealed clear differences between them (Fig. 2C), with the first two principal components explaining 65.5% of the variability. Variability along PC1 was mainly explained by the opposition between stations with increasing salinity values and decreasing concentration of phosphates, nitrates, nitrites and ammonium. Differences between the two systems were clear through their separation along PC2. In fact, variability along PC2 was essentially explained by the opposition between the higher proportion of OM content and the higher proportion of silt+clay in bottom sediments observed in the Mira estuary, and higher proportion of mean sand in the Mondego.

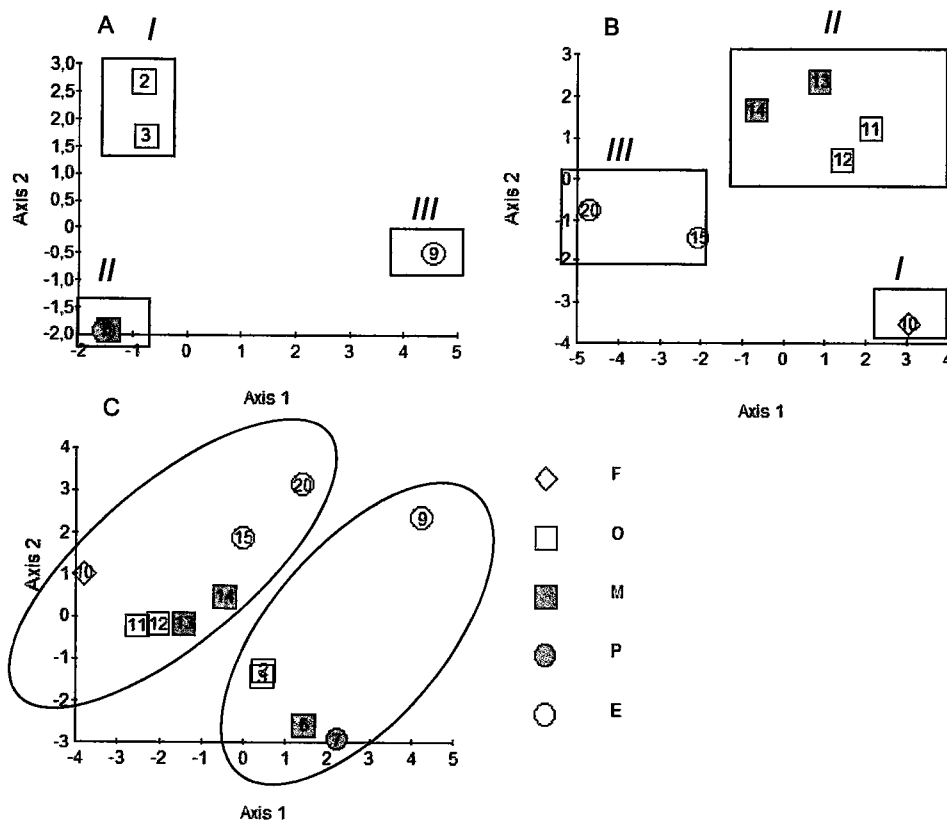


Figure 2 - Principal component analysis (PCA) plot based on the abiotic parameters at each station from A. Mira estuary (Axis1=51.1%, Axis2=34.2%), B. Mondego estuary (Axis1=55.5%, Axis2=32.3%) and C. Mira and Mondego estuaries simultaneously (Axis1=38.3%, Axis2=27.3%). F. Freshwater; O. Oligohaline; M. Mesohaline; P. Polyhaline and E. Euhaline.

Nematode community characteristics

At Mira estuary a total of 45 nematode genera of 19 families were identified. The most common families were Comesomatidae (25.7%), Chromadoridae (15.0%) followed by Leptolaimidae (13.9%), Desmodoridae (13.2%), Xyalidae (9.2%) and Anoplostomatidae (6.3%). All other families represented relative abundances lower than 4.5%. Genera with highest relative densities were *Sabatieria* (24.5%), *Ptycholaimellus* (13.8%), *Metachromadora* (13.2%), *Terschellingia* (12.8%), *Daptonema* (9.2%), *Anoplostoma* (6.3%) and *Sphaerolaimus* (4.5%), representing 84.3% of the total nematode density. A complete list of all identified nematodes genera and their abundances at both estuaries in each sampling station is shown in Tables 3 and 4. In every sampled station *Anoplostoma*, *Daptonema*, *Sabatieria*, *Terschellingia* and *Viscosia* genera were present. The highest numbers of genera, 33, were collected in euhaline section (station 9), 17 being limited to this sampling station. On the other hand, at oligohaline station 2 the lowest genera number was collected (10), *Oxystomina* and *Prochromadorella* genera being restricted to this section of the estuary.

At Mondego estuary a total of 48 nematode genera of 19 families were identified. The most common families were Desmodoridae (19.3%), Anoplostomatidae (13.6%), Xyalidae (10%), Comesomatidae (9.8%), Chromadoridae (8.6%), Microlaimidae (8.2%), Linhomoeidae (4.9%), Sphaerolaimidae (4.3%) and Axonolaimidae (4.1%). All other families contributed less than 3.1% to nematode abundance. Genera with highest relative densities were *Metachromadora*, (19.3%), followed by *Anoplostoma* 13.6% *Daptonema* (9.8%), *Sabatieria* (9.8%), *Microlaimus* (8.1%) and *Sphaerolaimus* (4.3%), *Axonolaimus* (3.8%), the freshwater *Dorylaimus* (3.4%), *Prochromadorella* (2.8%), *Dichromadora* (2.8%) and *Viscosia* (2.6%), representing 80.3 % of the total nematode density. In every sampled station only *Daptonema* genus was present along the estuary. At southern arm, the highest number of genera was collected (29 genera), 8 of them being limited to this sampling station. At freshwater station, the lowest number of genera was registered (10 genera), being *Monhystera*, *Stygodesmodora* and *Syringolaimus* exclusive of this section of the estuary (Table 4).

The average nematode density was higher in the Mira estuary ($603.3 \text{ ind.}10\text{cm}^{-2}$) than in the Mondego ($311.0 \text{ ind.} 10\text{cm}^{-2}$) but the number of genera in every salinity range was higher in the Mondego estuary.

At Mira system the mean nematode density fluctuated between $109.0 \pm 26.7 \text{ ind.} 10\text{cm}^{-2}$ at oligohaline station (station 3) and $2234.0 \pm 400.2 \text{ ind.} 10\text{cm}^{-2}$ at polyhaline section (station 7). With regard to nematodes densities, significant differences between stations ($F=30.62$, $p<0.05$) (Fig 3A) were obtained due higher density at station 7, although the remaining stations did not present differences between them (SNK). At Mondego estuary, the mean nematode density fluctuated between $38.9 \pm 5.3 \text{ ind.} 10\text{cm}^{-2}$ at freshwater section (station 10) and $1323.1 \pm 398.5 \text{ ind.} 10\text{cm}^{-2}$ at euhaline section (station 20). Nematodes densities registered significant differences between stations ($F=12.03$, $p<0.05$) (Fig 3B) due higher density obtained at station 20, although between the remaining stations there were not found differences (SNK).

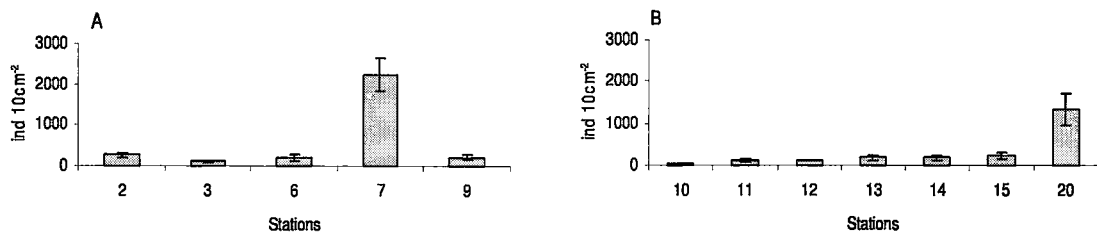


Figure 3 – Mean density \pm SE of nematodes (ind 10cm^{-2}) in each one of the sampling station in A- Mira estuary and B- Mondego estuary.

Table 3. Mean density (number of individuals 10cm⁻²) and standard error (\pm SE), of each nematode genera in the sampled stations in the Mira estuary, and respective trophic group.

Genera	Total	Trophic group	2		3		6		7		9	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>Sabatieria</i>	738.67	1B	1.23	1.23	0.48	0.48	76.87	55.98	636.64	237.66	23.45	14.79
<i>Ptycholaimellus</i>	417.14	2A			0.48	0.48	2.41	2.41	405.87	134.05	8.37	4.53
<i>Metachromadora</i>	397.25	2B					2.44	1.22	388.49	216.21	6.32	3.20
<i>Terschellingia</i>	384.55	1A	130.17	75.67	48.79	16.15	29.44	7.46	166.82	81.95	9.33	4.56
<i>Daptonema</i>	277.38	1B	3.29	0.67	1.04	0.53	22.23	6.96	222.63	99.26	28.18	7.57
<i>Anoplostoma</i>	189.64	1B	3.43	1.84	7.28	3.73	26.41	12.51	152.20	58.74	0.32	0.32
<i>Sphaerolaimus</i>	137.02	2B			0.48	0.48	22.40	10.05	113.50	54.68	0.64	0.64
<i>Thalassironus</i>	88.31	2B							86.46	39.85	1.85	1.85
<i>Paracyntholaimus</i>	33.93	2A	15.26	4.39	9.68	6.71	2.88	0.56			6.11	4.79
<i>Viscosia</i>	26.18	2B	2.45	2.45	0.57	0.28	10.37	2.70	10.10	5.41	2.68	1.50
<i>Linhomoeus</i>	25.43	2A					0.60	0.60			24.83	19.56
<i>Axonolaimus</i>	24.30	1B	3.88	2.30	8.09	5.81			12.33	12.33		
<i>Paracomesoma</i>	22.13	1B									22.13	7.69
<i>Odontophora</i>	21.18	1B									21.18	12.64
<i>Dichromadora</i>	18.42	2A							16.60	16.60	1.82	0.91
<i>Synonchiella</i>	16.72	2B									16.72	6.26
<i>Metacomesoma</i>	11.42	1B									11.42	10.95
<i>Aegialolaimus</i>	8.30	1A							8.30	8.30		
<i>Spilophorella</i>	7.88	2A							7.88	7.88		
<i>Ascolaimus</i>	6.45	1B			0.28	0.28			6.17	6.17		
<i>Comesoma</i>	3.46	1B									3.46	3.46
<i>Chromadorella</i>	2.37	2A									2.37	1.51
<i>Metalinhomoeus</i>	2.35	1B	1.23	1.23			0.26	0.26			0.86	0.86

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Table 3 (continued)

Genera	Total	Trophic group	2		3		6		7		9	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>Diodontolaimus</i>	2.13	2A			0.56	0.56					1.56	0.82
<i>Prochromadorella</i>	2.10	2A	2.10	2.10								
<i>Halalaimus</i>	1.84	1A			1.05	0.53	0.78	0.78				
<i>Hypodontolaimus</i>	1.82	2A					0.26	0.26			1.56	0.82
<i>Spirinia</i>	1.82	2A									1.82	0.91
<i>Antomicron</i>	1.47	1A					1.47	1.10				
<i>Leptolaimus</i>	1.25	1A			1.25	0.86						
<i>Camacolaimus</i>	1.23	2A					1.23	1.23				
<i>Eurystomina</i>	1.23	2B					1.23	1.23				
<i>Oxystomina</i>	1.23	1A	1.23	1.23								
<i>Monhystera</i>	0.93	1B									0.93	0.93
<i>Odontanticoxa</i>	0.93	1A									0.93	0.93
<i>Anticoxa</i>	0.86	1A									0.86	0.86
<i>Oncholaimellus</i>	0.86	2B									0.86	0.86
<i>Oncholaimus</i>	0.86	2B									0.86	0.86
<i>Prochromadora</i>	0.86	2A									0.86	0.86
<i>Chromadorita</i>	0.60	2A			0.28	0.28					0.32	0.32
<i>Aponema</i>	0.32	1A									0.32	0.32
<i>Chomadorina</i>	0.32	2A									0.32	0.32
<i>Chromadora</i>	0.32	2A									0.32	0.32
<i>Cyatholaimus</i>	0.32	2A									0.32	0.32
<i>Wieseria</i>	0.29	1A			0.29	0.29					0.32	0.32
Freshwater nematodes	132.41	-	103.26	30.03	28.36	11.81	0.78	0.78				

Table 4. Mean density (number of individuals 10cm⁻²) and standard error (\pm SE), of each nematode genera in the sampled stations in the Mondego estuary, and respective trophic group.

Genera	Total	Trophic group		10		11		12		13		14		15		20		
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
<i>Metachromadora</i>	419.36	2B															418.76	140.25
<i>Anoplostoma</i>	296.67	1B		0.81	0.41	19.83	6.02	23.11	7.80	118.70	42.46	1.94	1.03	132.28	37.99			
<i>Daptonema</i>	213.31	1B	0.84	0.19	27.34	18.61	20.80	3.61	115.87	37.78	10.55	2.94	25.01	11.48	12.89	3.36		
<i>Sabatieria</i>	212.55	1B		0.77	0.39	0.71	0.71	0.71	0.71	1.62	1.62	116.89	52.40	92.57	37.26			
<i>Microaimus</i>	176.11	2A	0.20	0.20	0.34	0.34	0.67	0.67	0.50	0.50	5.18	2.30	6.71	2.28	79.84	33.17		
<i>Sphaerolaimus</i>	92.90	2B		0.38	0.38	9.94	5.42	2.79	0.34	6.10	2.48	0.76	0.76	62.88	38.63			
<i>Axonolaimus</i>	82.09	1B		3.66	2.75	10.21	5.85	7.18	3.82	4.10	1.22	20.99	7.64	13.77	10.76			
<i>Prochromadorella</i>	61.30	2A		0.41	0.21	0.33	0.33	0.33	0.33	2.85	2.85	2.99	1.71	22.07	3.83			
<i>Dichromadora</i>	59.92	2A		5.06	3.50	0.43	0.43	4.12	2.07	0.54	0.54	14.46	6.64	25.62	17.62			
<i>Viscosia</i>	56.56	2B	0.41	0.21	2.71	1.11	29.43	4.84	2.99	1.71	7.23	4.56	7.95	5.11				
<i>Paracyatholaimus</i>	55.36	2A		0.21	0.21	4.83	2.43	4.05	2.43	6.47	6.47	22.75	9.96	4.95	4.95			
<i>Terschellingia</i>	45.18	1A		0.44	0.44					0.71	0.71			34.97	34.97			
<i>Leptolaimus</i>	43.27	1A												29.50	13.31			
<i>Calyptonema</i>	34.97	2B												29.69	29.69			
<i>Chromadora</i>	30.54	2A												26.50	13.54			
<i>Paralinhomoeus</i>	30.40	1B												20.84	7.09			
<i>Aegialobalaimus</i>	26.50	1A												19.08	13.35			
<i>Linhomoeus</i>	23.88	2A												9.90	9.90			
<i>Halalaimus</i>	19.08	1A												7.06	4.30			
<i>Southemiella</i>	12.99	1A	0.20	0.20						1.21	0.63	1.08	1.08	9.90	9.90			
<i>Ptycholaimellus</i>	11.33	2A		3.50	2.21	0.34	0.34	0.34	0.34	0.42	0.42			9.90	9.90			
<i>Praeacanthorchus</i>	9.90	2A												9.90	9.90			
<i>Hypodontolaimus</i>	8.96	2A												1.82	1.40			
<i>Camacolaimus</i>	8.67	2A	0.21	0.21	0.81	0.41	1.35	0.89	4.43	7.61	4.43			5.83	5.83			

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Table 4 (continued)

Genera	Total	Trophic group	10		11		12		13		14		15		20	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>Chromadorita</i>	8.65	2A	0.20	0.20					3.20	2.22			0.30	0.30	4.95	4.95
<i>Ascolaimus</i>	6.46	1B					5.60	2.30	0.86	0.44						
<i>Desmolaimus</i>	4.95	1B													4.95	4.95
<i>Chromadorina</i>	4.48	2A			0.81	0.41	2.65	1.32	0.72	0.72			0.30	0.30		
<i>Oncholaimellus</i>	3.47	2B			0.44	0.44			0.50	0.50	0.42	0.42			2.12	2.12
<i>Cobbia</i>	2.35	2A			1.02	0.56	1.34	1.34								
<i>Aponema</i>	2.12	1A													2.12	2.12
<i>Araeolaimus</i>	2.12	1A													2.12	2.12
<i>Eumorpholaimus</i>	2.12	1B													2.12	2.12
<i>Paracomesoma</i>	1.39	1B			0.38	0.38			0.71	0.71			0.30	0.30		
<i>Cromadorella</i>	1.18	2A											1.18	1.18		
<i>Doliolaimus</i>	1.05	2B					0.33	0.33	0.71	0.71						
<i>Paramonhystera</i>	1.03	1B					0.43	0.43					0.60	0.60		
<i>Stygodesmodora</i>	1.02	2B	1.02	0.54												
<i>Spilophorella</i>	1.01	2A					1.01	1.01								
<i>Tripyloides</i>	0.76	1B					0.34	0.34			0.42	0.42				
<i>Marylynnia</i>	0.71	2A							0.71	0.71						
<i>Paracanthonchus</i>	0.64	2A					0.34	0.34					0.30	0.30		
<i>Monhystera</i>	0.62	1B	0.62	0.62												
<i>Valvaelaimus</i>	0.43	2A					0.43	0.43								
<i>Odontophora</i>	0.42	1B									0.42	0.42				
<i>Comesoma</i>	0.34	1B					0.34	0.34								
<i>Diplolaimella</i>	0.30	1B											0.30	0.30		
<i>Syringolaimus</i>	0.20	2B	0.20	0.20												
Freshwater nematodes	97.19	-	32.31	2.88	56.08	33.04	4.65	0.38	1.49	1.49	0.54	0.54	0.30	0.30	2.12	2.12

Nematoda community patterns

The MDS analysis, using nematode data from the three replicates collected at each station in both systems, indicated that replication was good since the stress values were low (0.08 in Mira and 0.1 in Mondego estuary).

The MDS plot based on data from Mira clearly reflected the nematodes spatial distribution along the salinity gradient (Fig. 4A). This MDS plot allowed recognizing distinct assemblages, according the salinity ranges of the estuary: *i*) Oligohaline section, included assemblages from stations 2 and 3, *ii*) Mesohaline section was represented by the community collected at station 6, *iii*) Polyhaline section by the community of station 7 and *iv*) Euhaline section, included community from station 9. The SIMPER analysis showed the highest dissimilarities between assemblages from the oligohaline and polyhaline sections (93.8%), due the presence of the freshwater nematode *Dorylaimus* in oligohaline section. The presence of *Paracomesoma*, *Synonchiella* and *Odontophora* in the upstream section and the highest densities of *Sabatieria*, *Ptycholaimellus*, *Metachromadora* and *Daptonema* had a contribution of 93.1% for the dissimilarity between the estuarine euhaline and polyhaline sections, while mesohaline section was characterised by the presence of *Terschellingia*, *Sabatieria*, *Daptonema* and *Anoplostoma* (Table 5A).

The BIOENV analysis showed that a combination of four variables (salinity, % of gravel, % of coarse sand and sediment organic matter) explained 89% of the variability found in the nematode community. In addition, nematode genera that characterised each assemblage were significantly correlated with those environmental factors (Table 6).

As for the Mira, nematode data from Mondego estuary reflected their spatial distribution along the salinity gradient (Fig. 4B). The MDS plot allowed the recognition of distinct assemblages according to the salinity ranges: *i*) Freshwater estuarine section, included community from station 10, *ii*) Oligohaline and Mesohaline estuarine sections included communities from stations 11 to 14, and *iii*) Euhaline section of the Northern arm included community from station 15 and *iv*) Euhaline section of the southern arm, included community from station 20 (Fig. 4B). The SIMPER analysis showed the highest dissimilarities between assemblages from the freshwater section and both the euhaline sections (southern arm, 98.9%; northern arm, 98.6%). The freshwater estuarine section was mostly characterised by freshwater nematodes and the southern arm registered the highest densities of *Metachromadora*, *Anoplostoma* and *Microlaimus*, while *Sabatieria*, *Leptolaimus* and *Dichromadora*, reached the highest densities in the northern arm (Table 5B).

The BIOENV analysis showed that a combination of four variables (% fine sand, PO_4^{3-} , NO_2^- and NH_4^+) explained 96% of the variability found in the nematode community. In addition, genera that typified each assemblage were significant correlated with salinity and water nutrients (Table 6).

The MDS plot resulting from the joint analysis of both estuarine systems also allowed the identification of distinct assemblages, according to the salinity ranges (4C):
i) Freshwater and Oligohaline sections, characterised by the presence of freshwater nematodes, by the lowest density (38.9-109.0 ind. 10cm^{-2}) and diversity (10-15 genera);
ii) Mesohaline sections included communities with low density (117.4-228.8 ind. 10cm^{-2}) and relatively low diversity (15-24 genera), the dominant nematode genera were *Terschellingia*, *Sabatiera*, *Daptonema* and *Anoplostoma* and
iii) Polyhaline and Euhaline sections, characterised by the highest nematode densities (204.0-2234.0 ind. 10cm^{-2}) and diversity (14-33 genera). *Paracomesoma*, *Synonchiella*, *Odontophora*, *Sabatiera*, *Metachromadora* *Daptonema* and *Ptycholaimellus* attained the highest densities in this section.

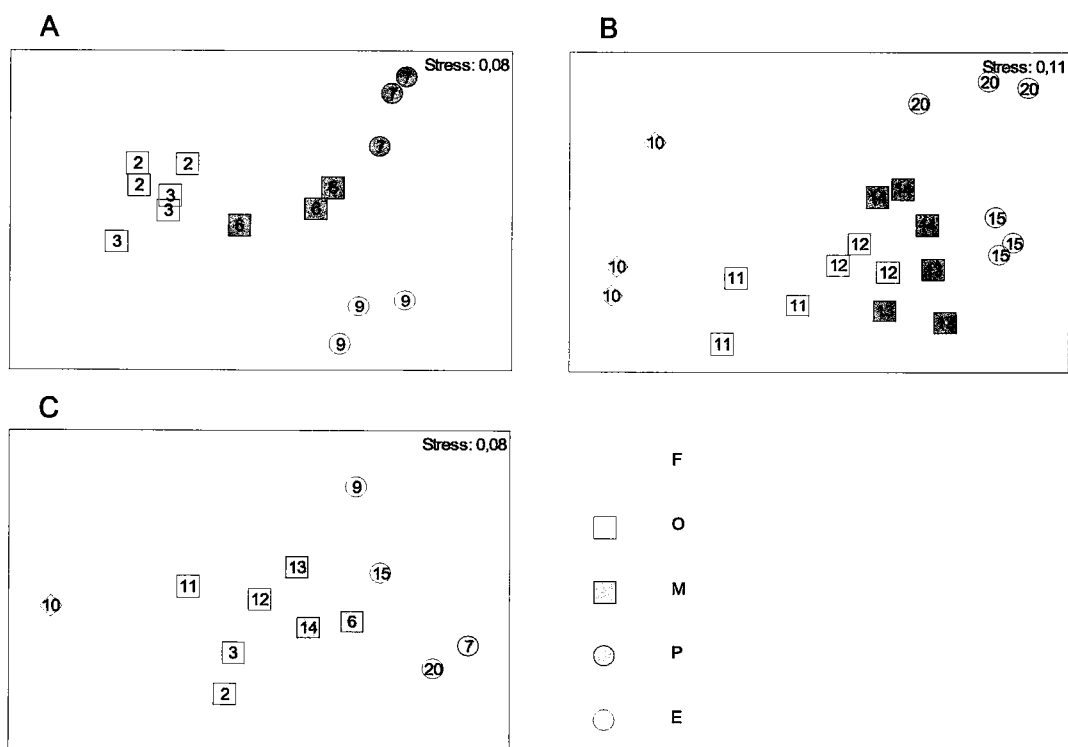


Figure 4 – Non-metric multidimensional scaling (MDS) plot based on the nematode density and composition from sampling stations in A- Mira estuary, B- Mondego estuary and C- Mira and Mondego estuary simultaneously. F. Freshwater; O. Oligohaline; M. Mesohaline; P. Polyhaline and E. Euhaline.

Table 5. Genera determined by SIMPER as those most responsible for contributing for the similarity within each group (group determined by MDS) for the Mira estuary and the Mondego estuary. Shaded boxes: percentage of similarity (bold) and genera that contributed for similarity between stations in each group. Non-shaded box, percentage of dissimilarity (bold) between two groups and percentage of dissimilarity that each genera contributed for the total dissimilarity

A. Mira estuary	Group I (st 2 and 3)	Group II (st 6)	Group III (st 7)	Group IV (st 9)
Group I	47.3%			
	<i>Terschellingia</i>			
	<i>Dorylaimus</i>			
	<i>Paracyatholaimus</i>			
Group II	76.1%	44.5%		
	<i>Sabatieria</i>	<i>Terschellingia</i>		
	<i>Dorylaimus</i>	<i>Sabatieria</i>		
	<i>Terschellingia</i>	<i>Daptonema</i>		
	<i>Anoplostoma</i>	<i>Anoplostoma</i>		
Group III	93.8%	84.4%	49.5%	
	<i>Sabatieria</i>	<i>Sabatieria</i>	<i>Sabatieria</i>	
	<i>Ptycholaimellus</i>	<i>Ptycholaimellus</i>	<i>Ptycholaimellus</i>	
	<i>Metachromadora</i>	<i>Metachromadora</i>	<i>Metachromadora</i>	
	<i>Daptonema</i>	<i>Daptonema</i>	<i>Daptonema</i>	
Group IV	91.2%	72.7%	93.1%	38.0%
	<i>Terschellingia</i>	<i>Sabatieria</i>	<i>Sabatieria</i>	<i>Daptonema</i>
	<i>Dorylaimus</i>	<i>Terschellingia</i>	<i>Ptycholaimellus</i>	<i>Paracomesoma</i>
	<i>Daptonema</i>	<i>Anoplostoma</i>	<i>Metachromadora</i>	<i>Synonchiella</i>
	<i>Paracomesoma</i>	<i>Paracomesoma</i>	<i>Daptonema</i>	<i>Sabatieria</i>
B. Mondego estuary	Group I (st10)	Group II (st 11-14)	Group III (st 15)	Group IV (st 20)
Group I	36.7%			
	<i>Dorylaimus</i>			
	Order Mononchida			
Group II	89.9%	29.7%		
	<i>Daptonema</i>	<i>Daptonema</i>		
	<i>Anoplostoma</i>	<i>Anoplostoma</i>		
	<i>Dorylaimus</i>	<i>Paracyatholaimus</i>		
	Fam Dorylaimidae			
Group III	98.6%	82.3%	48.8%	
	<i>Sabatieria</i>	<i>Sabatiera</i>	<i>Sabatieira</i>	
	<i>Leptolaimus</i>	<i>Anoplostoma</i>	<i>Leptolaimus</i>	
	<i>Daptonema</i>	<i>Daptonema</i>	<i>Dichromadora</i>	
	<i>Dichromadora</i>	<i>Leptolaimus</i>		
Group IV	98.9%	89.3%	84.8%	50.9%
	<i>Metachromadora</i>	<i>Metachromadora</i>	<i>Metachromadora</i>	<i>Metachromadora</i>
	<i>Microlaimus</i>	<i>Microlaimus</i>	<i>Microlaimus</i>	<i>Anoplostoma</i>
	<i>Anoplostoma</i>	<i>Anoplostoma</i>	<i>Anoplostoma</i>	<i>Microlaimus</i>
	<i>Sabatieria</i>	<i>Sabatieria</i>	<i>Sabatieria</i>	<i>Sabatieria</i>

Table 6 - Significant correlations (*r*) between the abundance of the nematode genera that typified each nematoda community in distinct salinity ranges.

	Genera	Salinity	Gravel	Coarse sand	Mean sand	Silt+Clay	PO ₄ ³⁻	NO ₃ ⁻	NO ₂ ⁻	NH ₄ ⁺
Mira	<i>Terschellingia</i>				-0.9*		0.9*			
	<i>Dorylaimus</i>	-0.97**	0.9*							
	<i>Sabatieria</i>			-0.9*						
	<i>Anoplostoma</i>					0.9*			0.9*	0.9*
	<i>Paracyatholaimus</i>					-0.9*				
Mondego	<i>Anoplostoma</i>	0.79*					-0.77*	-0.08*	-0.78*	-0.78*
	<i>Metachromadora</i>	0.80*			0.8*		-0.81*	-0.81*	-0.81*	-0.81*
	<i>Sabatieria</i>	0.92**					-0.95**	-0.95**	0.95**	-0.95**
	<i>Leptolaimus</i>	0.86*					-0.88**	-0.88**	0.88**	-0.88**
	<i>Dichromadora</i>	0.82*					-0.85*	-0.85*	-0.85*	-0.85*
	Order	-0.80*					0.81*	0.81*	0.81*	0.81*
	Mononchida									

*, p<0.05;

**, p<0.01

Nematode feeding groups

In the Mira estuary, a clear dominance of non-selective deposit feeders (1B: 45%, 11 genera) was observed, followed by predators (2B: 23.2%, 8 genera), epigrowth-feeders (2A: 17.9%, 16 genera) and, with less density, selective deposit feeders (1A: 13.9%, 10 genera). In the oligohaline section, selective deposit feeders (1A) dominated, followed by non-selective deposit feeders (1B); in the mesohaline and polyhaline sections, non-selective deposit feeders (1B) dominated, followed by predators (2B), while in the euhaline section, non selective deposit feeders (1B) dominated, followed by epigrowth-feeders (2A) (Fig. 5A)

The spatial distribution patterns of the feeding types along salinity gradient was analysed at both estuarine systems. Although the patterns were different for both estuaries, it was possible to define distinct feeding types assemblages, according to the salinity ranges (Fig. 6).

The MDS analysis based on the percentage of contribution of each feeding type allowed separating the trophic structure of nematodes communities of the oligohaline section from the other estuarine sections (Fig. 6A). The SIMPER analysis showed that the dissimilarities observed (60%) between assemblages from the oligohaline and the remaining salinity estuarine ranges were obtained because of the higher abundance of selective deposit feeders (1A) in the oligohaline section and higher abundance of non-

selective deposit feeders (1B), predators (2B) and epigrowth-feeders (2A) in the remaining sections

In the Mondego estuary, non-selective deposit feeders (1B: 41.0%, 15 genera) and predators (2B: 29.3%, 8 genera) were the most abundant feeding type, followed by epigrowth-feeders (2A: 22.4%, 18 genera), while the selective deposit-feeders (1A: 7.3%, 7 genera) contributed with relative less density. In the freshwater section, a clear dominance of epigrowth feeders (2A) was observed, while in the remaining sections, excluding the euhaline section of the southern arm where predators dominated, non-selective deposit feeders (1B) was the most abundant feeding group (Fig. 5B).

^A The MDS analysis allowed to describe three distinct groups, that reflected the spatial distribution of the trophic structure of the communities along the salinity gradient (Fig. 6B): (1) freshwater and euhaline sections of the south arm; (2) oligohaline section and (3) mesohaline and euhaline section of the northern arm (Fig. 6B). The SIMPER analysis showed the highest dissimilarities (53%) between assemblages from freshwater and euhaline section of the south arm (1) and mesohaline and euhaline section of the northern arm (3) due mainly to higher density of non-selective deposit feeders (1B) in this last section and higher density of epigrowth-feeders (2A) and predators (2B) in the freshwater and euhaline section of the southern arm (1).

The MDS analysis of the communities from both estuaries based on their feeding type composition allowed a clear separation of stations from the two systems, especially the oligohaline stations of the Mira estuary that were completely apart from the remaining ones (Fig. 6C).

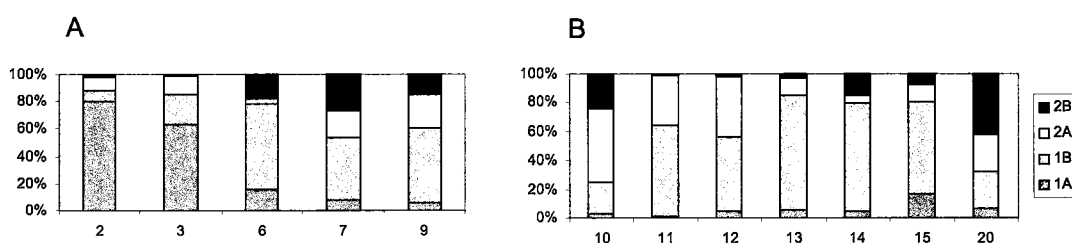


Figure 5 - Percentage of contribution of each feeding group in each of the sampled stations in the A- Mira and B- Mondego estuary. 1A, selective deposit feeders; 1B, non-selective deposit feeders; 2A, epistrate feeders; 2B, predators (Wieser, 1953).

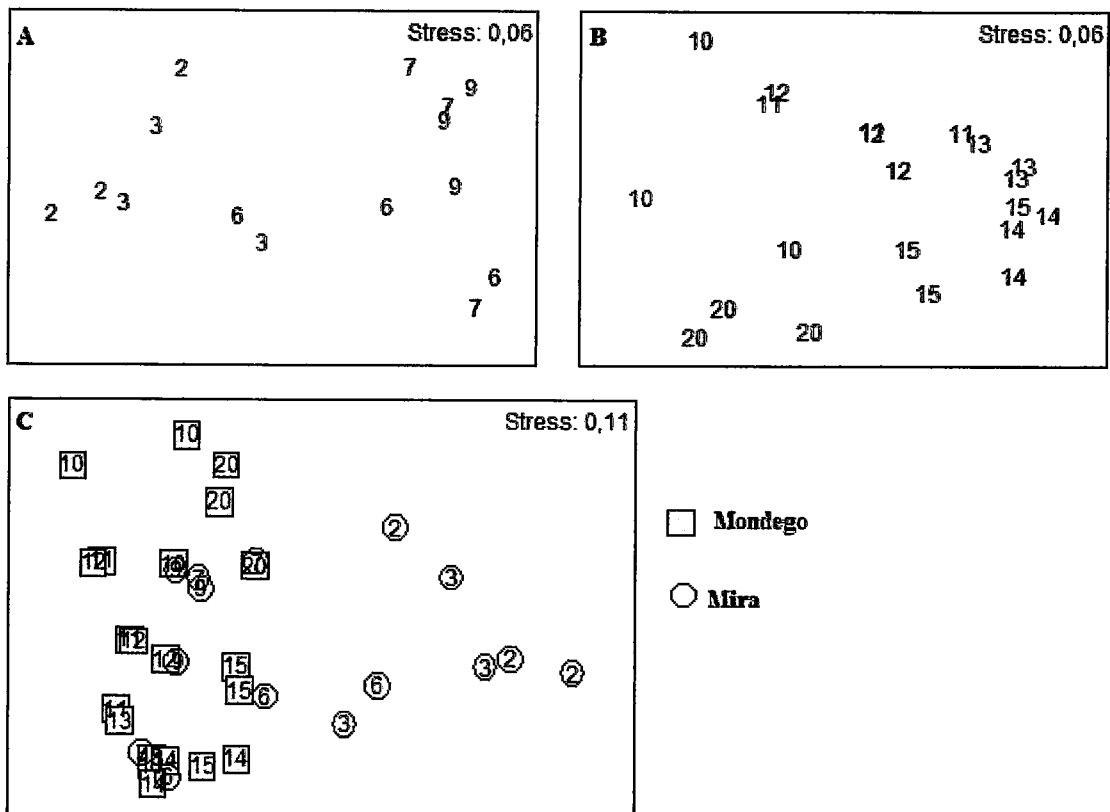


Figure 6 - Non-metric multidimensional scaling (MDS) plot based on the percentage of contributing of each feeding groups from sampling stations in A- Mira estuary, B- Mondego estuary and C- Mira and Mondego estuary simultaneously.

DISCUSSION AND CONCLUSIONS

Gradients of salinity and sediment particles size were clearly detected at both estuaries.

In the Mira, the observed salinity gradient is mostly explained by the morphology of the estuary - a single river channel and an almost absence of irregularities in its terminal section - which allows the tidal influence to extend about 40 km inland (Paula et al., 2006). In the Mondego, due to the distinct hydrological regimes of the northern and southern arms, two salinity gradients are recognizable. The northern arm is deeper and has been heavily modified, namely in the last two decades, by the construction of stonewalls along the river banks and of small water reservoirs for aquaculture, which caused changes in hydrodynamics and had a strong anthropogenic impact. The tidal penetration is therefore faster along the northern arm and salinity is higher than in the southern arm during high-water periods. On the contrary, the south arm is much shallower, almost constituting a kind of coastal lagoon in which the water circulation depends mostly on the tides and on the freshwater input on the irregular discharge, controlled by a sluice, from a small tributary, the Pranto River (Cardoso et al., 2004).

A typical gradient of estuarine sediments was observed in the Mira, with fractions of silt and clay increasing from the upstream sections towards the mouth of the estuary. The only exception was sampling station 9 (sediments consisting of 90% sand), very close to the mouth, due to its location on top of a sand flat. On the contrary, the gradient of estuarine sediments was much less clear in the Mondego estuary. In general, in the Mondego estuary, subtidal bottoms presented larger fine sand fractions and much smaller silt + clay fractions than in the Mira. Moreover, sediments distribution pattern was found to be unlike in the two arms (Teixeira et al., 2007).

In the Mira, nutrients concentrations did not show any spatial pattern of variation during the sampling period, remaining constant along the estuary, which may be explained by the absence of significant inputs related with anthropogenic activities. On the contrary, in the Mondego, nutrients concentrations [ammonium (NH_2), the oxidised forms of nitrogen, and phosphates] were higher in the northern arm than in the southern one. Actually, a previous study in the Mondego estuary suggested a strong dependency of the concentration of oxidised forms of dissolved nitrogen on the freshwater inputs from diffuse and/or point sources, which may include precipitation and the consequent freshwater flow with agricultural lands draining, as main sources of nitrate (Lillebø et al., 2007).

Nematodes density was higher in Mira estuary, although at each analogous salinity range in Mondego estuary the number of genera was higher and the rank of nematodes densities was similar of the communities studied in subtidal sediments of the northern European estuaries, although the number of genera were relatively lower (Smol et al., 1994; Soetaert et al., 1994). The subtidal nematode densities were lower than those reported for intertidal estuarine sediments that commonly present higher abundances and nematode diversity (Soetaert et al., 1994; 1995; Steyaert et al., 2003) than subtidal sediments.

At both estuaries, subtidal nematode communities showed a clear distribution pattern of the density, composition and feeding structure related with the salinity gradients. Assemblages composition closely resemble that of the north European estuaries, mainly composed by *Sabatieria*, *Metachromadora*, *Daptonema*, *Anoplostoma*, *Sphaerolaimus*, and *Terchellingia*, which are the most common genera for tidal estuarine mudflat, and due the dominance of few species, as recorded from other estuaries (Austen & Warwick, 1989; Li & Vincx, 1993; Soetaert et al., 1995; Steyaert et al., 2003; Rzeznik-Orignac et al., 2003).

For both estuaries the density and composition of the nematode assemblages followed closely the salinity gradients, with both nematode density and diversity increasing from freshwater and oligohaline sections to polyhaline and euhaline

sections. In fact, Soetaert et al. (1995) in a study of nematode communities in five European estuaries concluded that nematode diversity was positively correlated with salinity and that nematode density increased with increasing salinity. Also Austen & Warwick (1989) stated that nematode densities decrease towards upstream and since the preponderance of species in estuaries is marine, there is a decrease in species richness as one moves toward freshwater (Coull, 1999). This illustrates the role of salinity as an important independent factor in the determination of the nematode assemblages and in describing the changes of the total density and diversity (Coull, 1985; Vincx et al., 1990; Li & Vincx, 1993; Soetaert et al., 1995; Yamamuro, 2000).

In the present study, we could confirm the direct effect of salinity on estuarine nematode communities, although we also illustrated that other environmental factors, such as granulometry, nutrients concentration and sediments organic matter content was associated to the differences of the densities and diversity obtained between both estuaries. In fact, in Mondego estuary, the sandy sediments contributed to a decrease in density and an increase in diversity as a result of the wider range of microhabitats available for nematodes, compared to muddy sediments (Steyaert et al., 2003).

The relative proportion of each of the four nematode feeding guilds in a community depends on the quality and quantity of their food sources, which in turn is a reflex of the sediment composition (Moens & Vincx, 1997; Danovaro & Gambi, 2002). The differences observed in the environmental conditions, on a horizontal scale, between the two estuaries, were not only reflected in genera composition and abundance but also in the trophic composition of the nematodes communities. Indeed, between the two estuaries, nematodes feeding type composition patterns were remarkably different which can be attributed to different food availability. The high abundance of epistrate-feeders in the Mondego in almost all the sections of the estuary can be attributed to the high nutrient concentrations as well as to the high organic matter content, contrasting with the Mira estuary, where deposit feeders, bacteria consumers, predominated in all the salinity sections. The differences of the physicochemical conditions between northern and southern arms in the Mondego and between upstream and downstream sections of the both estuaries changed the nematode feeding composition as a response to food availability.

Subtidal nematode density and composition patterns are a reflection of both the sediments composition and hydrodynamic conditions. In the studied estuaries, the heterogeneity was much higher along the estuarine gradients than between estuaries. Consequently, mesoscale variability within estuaries (at the scale of km), due to salinity changes and grain size differences is often more important than variability at the scale of hundreds of km or between estuaries (Soetaert et al., 1995; Li et al., 1997).

In this study, the spatial variability was a reflex of the natural stressors characteristic of estuaries and the features of anthropogenic stressors of the Mondego estuary seems not relevant to nematode community patterns. Therefore, when attempting to determine the effects of human activities in nematode communities in estuaries it becomes a "problem", as defined by "The Estuarine Quality Paradox" (Dauvin, 2007). On the other hand, the different patterns of the proportion of trophic structure assemblages between Mira and Mondego outweighed the salinity effects (natural stressor) and the feeding guilds and their response could detect the anthropogenic-induced stress in estuaries. In this context, and as has been already stated (Frid et al., 2000; Bremner et al., 2003), taxonomic and functional analyses should complement each other when developing general descriptions of benthic diversity to a better understanding of the effects of environmental variables and human activities on the communities and a better knowledge of the functional roles of nematodes will be important to develop the sensitivity and interpretation of biological traits analyses of the communities (Schratzberger et al., 2007).

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Chapter 4

General conclusions and Final considerations

With the present work it was possible to understand that both meiofauna and Nematoda communities are being structured and influenced by salinity, which acts as a natural stressor. The distinct anthropogenic pressures between the estuaries seem to be corroborated by the proportion of the trophic composition.

Since Meiofauna is considered a good biological indicator for environmental monitoring, especially on the study of the effects of anthropogenic activities and pollution (Coull & Chandler, 1992; Kennedy & Jacoby, 1999), we were expecting to find differences between the two estuaries. However, there were not observed important differences, which may be related with the amount of analysed data, since only one season was analysed (Summer) and the seasonal variation of the communities could allow a better understanding of the meiofauna dynamics in these estuaries. In the same way, Nematoda communities are also recognized as able to detect the effects of anthropogenic activities (e.g. Boyd et al., 2000; Schratzberger et al., 2002, 2006; Gheskiere et al., 2005). Nevertheless, in the present study the composition and densities of Nematoda communities at both estuaries were not clearly different. This is an important fact to bear in mind when intending to use benthic indices based on species to assess the biological quality of the estuarine environment, since the responses to both anthropogenic and natural stress could not be easily differentiated as defined by "The estuarine Quality Paradox" (Dauvin 2007).

In what refers to the main environmental variables influencing not only meiofauna but also Nematoda communities, the abiotic factors were similar in both estuaries. This, and although we have analysed only a few set of data, can be important in terms of monitoring programs, since it may provide a significant reduction in costs due to the high taxonomic identification expertise, as has already been stated for other benthic communities (Ferraro & Cole, 1995; Pagola-Carte et al., 2002; Chainho et al, 2007). This concept of "taxonomic sufficiency" refers to taxonomic identification to the highest possible level that retains taxonomic accuracy and sufficient biological information to assess environmental stress effects and is based on the assumption that taxa can be identified to a taxonomic level higher than the species level without losing the ability to detect changes related to pollution stress (Chainho et al., 2007). But despite the advantages, taxonomic sufficiency is a controversial topic, since it might generate losses of ecological information (Maurer, 2000). In our specific approach the separation of the two estuaries based on their biotic communities, was achieved with the proportion of trophic composition of the nematode communities. In fact, studies on macrobenthic invertebrates have shown that linking taxonomic and

functional diversity (i.e. pooling species from different taxonomic entities into functionally similar groups) can reveal different relationships between assemblages (e.g. Bremner et al. 2003; Bostrom et al., 2006), and provide more information about the systems.

It is thus essential to have a good set of data such as seasonal and interannual comparisons of meiofauna and environmental characteristics, so that the described "capacities" of meiofauna and nematodes to be used as a monitoring tool can be useful in the Portuguese estuaries.

Salinity was a structuring abiotic factor in the distribution pattern of composition and density of meiobenthos and Nematoda assemblages. Nevertheless, sediment grain size was also a very important factor, partly covering the salinity effect, as we could observe with the meiofauna analyses and with the trophic nematode composition which was related with sediments properties.

This study represents the first set of available data for subtidal Meiobenthos and Nematoda communities in the Portuguese estuaries and the results of the present work serve as a base-line for posterior studies. Further studies are indispensable to better understand the dynamic of these communities, especially temporal series that would allow the identification of trends along the year for the achievement of a better knowledge of the communities, for the subsequent comprehension of the effects of extreme events over these communities.

Chapter 5

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