

# Spatial distribution of subtidal meiobenthos along estuarine gradients in two southern European estuaries (Portugal)

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*The spatial variation in abundance and composition of subtidal meiobenthos communities was studied in two Portuguese estuaries under distinct degrees of anthropogenic pressure. Samples were collected from 20 sampling stations along both estuarine gradients from freshwater (<0.5) to euhaline areas (>30). Of these stations 11 were located in the Mondego estuary, a system exposed to high anthropogenic stress, and 9 in the Mira estuary, a naturally stressed system located inside the Natural Park 'Costa Vicentina' (south-western coast of Portugal).*

*Total meiobenthos density was higher in the Mira estuary and in both estuaries the community was dominated by nematodes. Among the twelve taxa identified in the Mira estuary, Harpacticoid copepods and Polychaeta were the second and the third most abundant groups respectively. In the Mondego estuary, Polychaeta was the second while Harpacticoid copepods was the third most abundant group in the thirteen total taxa recorded.*

*Estuarine gradients of salinity, particles size and water nutrients were clearly detected in both estuaries and there was significant spatial heterogeneity in abundance and composition of the subtidal meiobenthos communities along these environmental gradients. Accordingly, three distinct sections were identified in both estuaries: (i) freshwater sections, where total density, and density of nematodes and Harpacticoid copepods reached minimum values; (ii) oligohaline and mesohaline sections, where the total density was low and diversity was poor; and (iii) polyhaline and euhaline sections, where both total density and diversity reached the highest values.*

*The similar spatial distribution of meiobenthos assemblages in both estuaries suggests that natural stressors may be the major factors controlling the physicochemical conditions that determine meiobenthos community patterns. The mesoscale variability of subtidal meiobenthos density and composition within estuaries (in the order of kilometres) seems to be more important than the variability between estuaries (at the scale of hundreds of kilometres), a common feature in intertidal systems. From the management point of view, these results, based on higher taxa resolution, represent an obvious constraint to the applicability of ecological quality evaluation tools.*

**Keywords:** subtidal meiofauna, estuarine gradients, natural versus anthropogenic disturbance, Portugal

Submitted 22 January 2008; accepted 23 February 2009; first published online 13 July 2009

## INTRODUCTION

Estuaries provide a natural gradient of salinity, often closely linked to other estuarine gradients, where abiotic conditions can change appreciably and continuously over a scale of kilometres. The importance of these abiotic factors (salinity, sediment grain size, sediment organic matter content, temperature, oxygen availability and pollution) in determining the temporal and spatial variation of meiofauna communities is well documented (Bouwman, 1983; Heip *et al.*, 1985; Austen & Warwick, 1989; Soetaert *et al.*, 1995; Li *et al.*, 1997; Forster, 1998; Moens & Vincx, 2000; Steyaert *et al.*, 2003; Derycke *et al.*, 2007).

The spatial patterns of meiofauna of estuarine and marine sediments have typically a strong spatially heterogeneous distribution and horizontal patchiness as a consequence of the natural variation in abiotic conditions along estuaries (Phillips & Fleeger, 1985). At larger scales (km), the meiofauna patchiness is commonly related to abiotic gradients in sediment composition and granulometry, salinity, temperature fluctuations and tide action (Li *et al.*, 1997). Physical factors are the main drivers of macroscale (km) heterogeneity in meiobenthos assemblages spatial patterns, while at the microscale (m) heterogeneity is caused by the effects of patchy food resources, predation, competition and reproduction behaviour (Sandulli & Pickney, 1999; Steyaert *et al.*, 2003).

A number of studies in several estuaries identified salinity as an important independent factor determining the structure of the meiobenthos communities (Soetaert *et al.*, 1995; Santos *et al.*, 1996). Salinity and sediment characteristics on the scale

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of hundreds of metres to kilometres were better predictors of community structure than latitudinal differences on the scale of hundreds of kilometres (Soetaert *et al.*, 1995). Likewise, it is well known that total meiobenthic density and number of species fall down as one moves from the sea towards freshwater (Austen & Warwick, 1989). As the preponderance of species in estuaries is marine, there is a decrease in species richness toward upstream (Coull, 1999). However, in some studies, the meiobenthos community distribution does not follow the salinity pattern. For example, species diversity in the Tamar estuary was lower in the mid-saline station than in upstream stations (Austen & Warwick, 1989). Although the salinity gradient is clearly important as a structural driver in estuaries, other factors can interact with and override the salinity effects (Austen & Warwick, 1989). Among these factors, the diameter of sediment particles is particularly important. For instance, in sediments with a higher detritus and clay content the meiofauna diversity decreases while abundance increases (Heip *et al.*, 1985). In another study, Coull (1985) described that abundance at a mud site was approximately twice that at a sand sediment site. Together, salinity/sediment-related factors explain many differences in meiofauna communities' distribution (Austen & Warwick, 1989; Soetaert *et al.*, 1995; Li *et al.*, 1997).

Apart from natural stressors, physical and chemical anthropogenic pressures can also modify the meiofauna pattern distribution. By altering the relative abundances of sensitive species, as well as their diversity and distribution patterns, anthropogenic pressures can be key factors influencing the structure and composition of meiobenthos communities (Essink & Keidel, 1998; Schratzberger & Warwick, 1998; Schratzberger *et al.*, 2004; Derycke *et al.*, 2007). Therefore, characterizing the distribution patterns of meiobenthic assemblages has become a useful biological tool to detect anthropogenic disturbance and environmental change (Warwick, 1981; Coull & Chandler, 1992). The identification of all organisms to the species level is very time-consuming and requires a high degree of taxonomic expertise and standardization. Some studies have shown that little information is lost by working at a taxonomic level higher than species, and at whatever taxonomic level the analysis is carried out, it is possible to obtain interpretable results if the community pattern changes markedly (Warwick, 1993; Somerfield & Clarke, 1995).

Although studies of meiofauna distribution are common at different spatial scales of estuarine intertidal environments, they have most often been carried out in a narrow range of estuarine gradients (Udalov *et al.*, 2005) and only a few of those included the oligohaline part of the salinity gradient (Soetaert *et al.*, 1994, 1995). Moreover, the knowledge concerning the spatial distribution patterns along estuarine gradients in subtidal estuarine ecosystems is comparatively scarce (Soetaert *et al.*, 1994; Smol *et al.*, 1994).

The structural characteristics of the meiobenthos estuarine communities have been well documented in several northern European estuaries (e.g. Ems—The Netherlands; Westerschelde—Belgium; Elba—Germany; Gironde—France; Tamar and Exe—UK). Nonetheless, there is a pronounced difference between the research efforts spent in the well studied northern systems and that spent in poorly known southern estuaries.

This study: (1) examined the spatial distribution patterns of subtidal meiobenthos communities along a wide range of estuarine gradients, particularly along the salinity gradient, in

southern European estuaries; (2) compared the meiofauna spatial patterns in two estuaries, one under 'light anthropogenic stress' and the other under 'high anthropogenic stress'; and (3) related the natural environmental factors with the meiofauna assemblages

The specific questions addressed in this study were:

- (1) How does the composition and density of meiofauna communities in subtidal sediments vary along the estuarine gradients in southern European estuaries?
- (2) Do distinct degrees of anthropogenic stress have an effect on meiofauna spatial distribution patterns along the estuarine gradients?
- (3) Is it possible to distinguish meiofauna spatial distribution patterns in the two estuaries under distinctly different degrees of anthropogenic stress, using higher taxonomic levels?

## MATERIALS AND METHODS

### Study areas

This comparative study was carried out simultaneously in the Mondego and Mira estuaries, both located on the western Atlantic coast of Portugal.

The Mondego estuary (40°08'N8°50'W) is a 21 km-long, warm-temperate intertidal system (Figure 1A). Its terminal part consists of two arms—northern and southern—separated by an alluvium island (Murraceira Island) and united again near the estuary's mouth. The two arms have very different hydrological characteristics. The southern arm is shallower (2–4 m during high tide), presenting large areas of intertidal mudflats (almost 75% of the area) exposed during low tide (Neto *et al.*, 2008). The northern arm is deeper (5–10 m during high tide) and receives most of the system's freshwater input, being strongly influenced by seasonal fluctuations in the water flow (Flindt *et al.*, 1997). In general, the Mondego estuary is under severe environmental stress (high anthropogenic stress) because it supports several industries and receives agricultural run-offs from rice and corn fields in the lower river valley (Lillebø *et al.*, 2007). Moreover, a harbour is located in the northern arm, where regular dredging is carried out to ensure appropriate shipping conditions. In the southern arm, clear eutrophication symptoms were observed in the early 1990s, particularly the occurrence of seasonal blooms of *Ulva* spp. and a concomitant severe reduction of the area occupied by *Zostera 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, as a function of the competition with macroalgae (Marques *et al.*, 2007). From 1998, several interventions were gradually undertaken, following the reduction in the freshwater discharge proceeding from the Pranto River sluice and the limited re-establishment of the upstream communication between the two arms (1997/1998), this trend appeared to reverse into a certain extent. A full re-establishment of the communication between the two arms was undertaken during the spring of 2006.

The Mira estuary (37°40'N 8°40'W) (Figure 1B), located on the south-west coast of Portugal, is 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, it is included in a protected zone—the Natural Park

of ‘Sudoeste Alentejano e Costa Vicentina’—and is therefore under light anthropogenic stress. This estuary is considered relatively undisturbed and free from industrial pollution (Costa *et al.*, 2001), and the physicochemical 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.

**Sampling**

Sampling was carried out 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) according to information gathered in earlier studies (Teixeira *et al.*, 2008). A total of nine stations in the Mira estuary (Stations 01, 1, 2, 3, 4, 6, 7, 8 and 11) and 11 stations in the Mondego estuary (Stations 25, 23, 21, 19, 18, 13, 12, 9, 7, 6 and 4) were selected, including the northern arm (Stations 13 and 12) and southern arm (Stations 9, 7, 6 and 4) subsystems of the Mondego estuary (Figure 1) (Table 1).

Three replicates of subtidal meiobenthos were collected at each sampling station by forcing a Kajak sediment corer (inner diameter: 4.6 cm) 3 cm into the sediment. All samples were preserved in 4% buffered formaldehyde. Meiofauna was extracted from the sediment fraction using Ludox HS-40 colloidal silica at a specific gravity of 1.18 g cm<sup>-3</sup> and using a 38 µm sieve (Vincx, 1996). All meiobenthic organisms were counted and identified at a higher

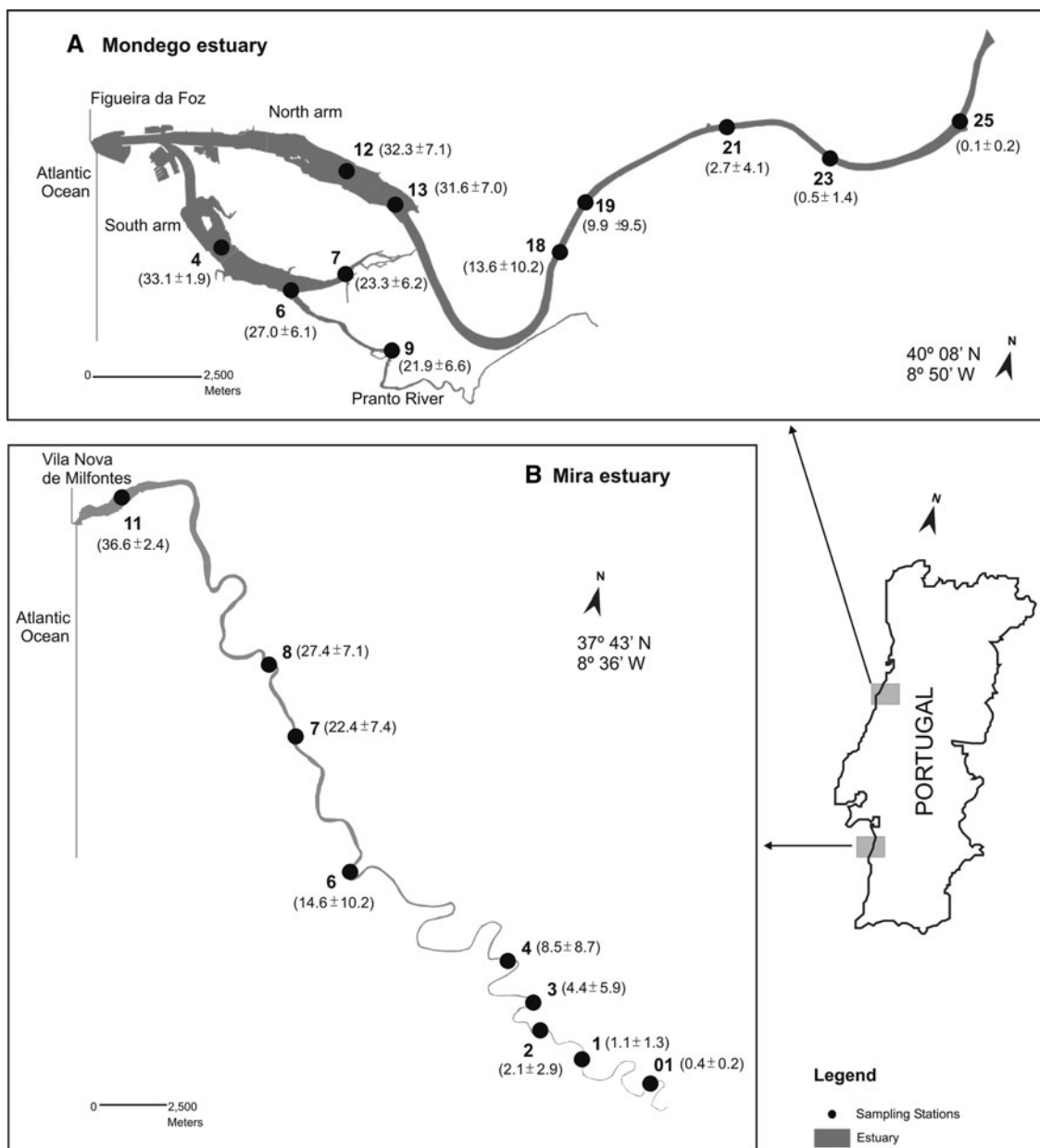


Fig. 1. Stations location (black circles) in (A) Mondego estuary and (B) Mira estuary. Bottom salinity values ± standard deviation (in parentheses) were based on earlier studies (Teixeira *et al.*, 2008).

**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	01	25
Oligohaline	0.5–5	1, 2, 3	23, 21
Mesohaline	5–18	4, 6	19, 18
Polyhaline	18–30	7, 8	9, 7, 6
Euhaline	>30	11	13, 12, 4

taxonomic level under a stereomicroscope. The abundance (individuals per 10 cm<sup>-2</sup>) of each meiofauna group was quantified. Meiofauna taxa identification was based on Higgins & Thiel (1988) and Giere (1993).

Water salinity, temperature, pH and dissolved oxygen (DO) (mg l<sup>-1</sup>) were measured *in situ*, during meiofauna sampling, using a Data Sonde Survey 4. Water nitrate (NO<sub>3</sub>) (μmol l<sup>-1</sup>) and nitrite (NO<sub>2</sub>) (μmol l<sup>-1</sup>) concentrations were analysed according to standard methods described in Strickland & Parsons (1972), and water ammonium (NH<sub>4</sub><sup>+</sup>) (μmol l<sup>-1</sup>) and water phosphate (PO<sub>4</sub><sup>3-</sup>) (μmol l<sup>-1</sup>) concentrations were analysed following the Limnologisk Metodik (1992) methodology. Sediment organic matter content (OM) was quantified by weight difference between the sediment weight after oven drying at 60°C for 72 hours and the sediment weight after combustion at 450°C for 8 hours, and expressed as a percentage of total weight. Grain size analysis was carried out by mechanical separation through a column of sieves with different mesh sizes. Sediment grain size was classified into five classes in accordance with Brown & McLachlan (1990): gravel (>2 mm), coarse sand (0.500–2.000 mm), 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 a percentage of the total sample weight.

## Data analysis

Data were analysed in order to: (a) characterize and compare taxa composition and density along the two estuaries; (b) compare the environmental variables influencing the structure and distribution of meiofauna assemblages in the two estuaries; and (c) relate the natural environmental factors with the meiofauna assemblages.

Differences in meiobenthos density between sampling stations at each estuary were tested by Kruskal–Wallis one-way analysis by ranks because assumptions for parametric statistics (e.g. normality and homogeneity of variance) were not met. Multivariate analysis was applied according to the procedures described by Clarke (1993). Non-metric multidimensional scaling (MDS) ordination using the Bray–Curtis similarity measure was applied to a square-root transformed matrix of the meiofauna data. Formal significance tests for differences between groups of samples were addressed using one-way ANOSIM tests (Clarke, 1993). The contribution of each taxon for the dissimilarity between groups of stations was determined by using the similarity percentage analysis procedure (SIMPER). The relationship between the environmental variables and the meiobenthic community structure was explored by carrying out a BIOENV analysis (Clarke & Ainsworth, 1993). A correlation-based principal components

analysis (PCA) using normalized Euclidean distance was applied to show spatial differences in environmental parameters.

All statistical analyses were performed using the software packages SPSS version 16 and PRIMER version 6 (Clarke & Warwick, 2001).

## RESULTS

### Abiotic factors

Abiotic variables measured along the estuarine gradients in the Mira and Mondego estuaries are shown in Table 2. Salinity gradients were observed in both systems with values increasing from upstream towards the estuaries' mouths (Figure 1).

A typical gradient of estuarine sediments was observed in Mira, where fractions of silt and clay increased from upstream to downstream. The only exception was the euhaline station 11, located at the estuary's mouth, with a higher percentage of sand (>90%). The three uppermost stations registered a predominance of coarse sediments (gravel + coarse sand > 64%). The other stations were characterized by sediments with a predominance of fine sand, silt and clay (>86%). The gradient of sediments in the Mondego estuary was much less clear, since the upstream section consisted predominantly of fine sand, with the exception of the uppermost station (Station 25), where the proportion of gravel and coarse sand was 82%. Distribution patterns of sediment composition were different between the two arms, with the northern arm being coarser and the southern arm being mostly composed of medium and fine sediment. Overall, the Mondego estuary had larger proportions of fine sand and much smaller proportions of silt + clay than the Mira estuary.

In both estuaries, the sediments characterized by a predominance of fine particles also presented the highest percentages of OM. In Mondego, the highest OM contents were obtained in sediments of the upper section (Station 18 with 4.8%) and in the Pranto station of the southern arm (Station 9 with 6.8%).

No spatial pattern was detected in nitrites and ammonium concentration in the Mira estuary (Table 2). The highest values were registered in Station 4 (1.357 μmol l<sup>-1</sup> and 9 μmol l<sup>-1</sup>, respectively), but remained constant throughout other stations in the estuary. In Mondego, on the contrary, the concentrations of ammonium, nitrates and phosphates were higher in the northern arm than in the southern arm, decreasing towards the mouth of the estuary in both arms.

Principal component analysis of abiotic parameters measured at each station of the Mira estuary identified three groups of sampling stations (Figure 2A): Group I— included oligohaline, mesohaline and polyhaline stations; Group II— included freshwater, oligohaline and mesohaline stations; and Group III— included the only euhaline station. The variability obtained was mainly due to higher proportions of silt + clay and OM in downstream stations and predominance of mean sand in upstream stations.

Likewise, PCA of Mondego's environmental factors also categorized the different stations into three major groups (Figure 2B): Group I— included polyhaline and euhaline stations located in the northern and southern arms; Group II— included oligohaline and mesohaline stations; and

**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 <sub>4</sub> <sup>3-</sup> (µmol/l)	NO <sub>3</sub> <sup>-</sup> (µmol/l)	NO <sub>2</sub> <sup>-</sup> (µmol/l)	NH <sub>4</sub> <sup>+</sup> (µmol/l)	OM (%)	Gr (%)	CS (%)	MS (%)	FS (%)	Silt + Clay (%)	
Mira	1	0.3	19.5	3.6	7.0	0.129	3.857	0.143	2.429	3.4	44.2	19.7	22.3	11.1	2.8	
	1	0.3	23.6	4.5	7.4	0.516	29.071	0.286	1.357	4.0	71.3	17.8	5.3	2.1	3.5	
	2	0.8	26.0	5.0	7.4	0.323	55.071	0.571	1.643	6.2	42.3	27.4	8.7	6.0	15.7	
	3	0.6	26.5	4.5	7.7	0.226	34.071	0.786	1.643	7.2	0.2	5.7	5.9	9.4	78.9	
	4	0.7	26.5	4.2	7.5	0.419	32.214	1.357	9.000	5.7	63.0	3.8	2.1	6.0	25.1	
	6	0.6	27.2	4.0	7.5	0.258	38.429	1.000	4.571	8.8	1.9	4.9	6.9	18.4	67.9	
	7	0.6	24.8	5.0	7.6	0.419	13.929	0.929	2.500	10.5	2.3	0.9	1.4	11.7	83.7	
	8	0.8	22.5	4.2	8.0	0.516	1.500	0.357	2.429	9.9	0.0	1.3	1.5	5.3	91.8	
	11	1.5	21.4	4.8	8.1	0.161	0.000	0.071	0.000	2.3	4.8	22.3	39.3	28.7	5.0	
	Mondego	25	0.6	24.0	6.4	7.4	3.097	95.071	4.286	13.143	0.2	35.8	46.0	16.2	1.9	0.2
		23	0.7	23.6	5.9	7.4	3.000	90.214	3.071	9.286	4.1	8.8	3.1	16.9	64.4	6.7
21		0.7	23.3	6.2	7.3	2.161	81.000	1.786	7.214	3.0	38.4	1.7	15.9	39.0	5.1	
19		1.1	22.8	6.2	7.3	2.161	81.000	1.786	7.214	3.8	0.2	0.9	14.4	74.1	10.4	
18		1.1	22.8	7.1	7.4	1.742	40.429	1.000	6.571	4.8	1.1	11.4	16.2	59.1	12.2	
13		2.8	19.0	7.6	7.5	1.452	26.571	0.714	4.714	1.4	29.7	26.3	22.0	17.5	4.5	
12		3.1	18.3	7.6	7.5	1.452	26.571	0.714	4.714	2.5	25.7	26.7	17.7	22.5	7.4	
9		0.5	23.4	8.6	7.8	1.000	6.286	0.286	3.286	6.8	0.2	0.9	25.1	68.7	5.1	
7		1.1	22.9	8.4	7.9	0.871	10.429	0.143	3.643	0.6	17.4	37.0	22.9	22.3	0.4	
6		2.0	20.7	7.9	7.9	1.419	22.286	0.500	6.571	1.4	11.8	35.2	23.9	26.6	2.6	
4		3.2	17.6	8.4	7.9	0.968	21.357	0.143	3.000	0.9	1.6	7.9	27.6	60.9	2.0	

Transp, transparency; T, temperature; DO, dissolved oxygen; PO<sub>4</sub><sup>3-</sup>, phosphate; NO<sub>3</sub><sup>-</sup>, nitrate; NO<sub>2</sub><sup>-</sup>, nitrite; NH<sub>4</sub><sup>+</sup>, ammonium; OM, sediment organic matter; Gr, gravel: >2 mm; CS, coarse sand: 0.5–2.0 mm; MS, mean sand: 0.25–0.50 mm; FS, fine sand: 0.063–0.250 mm; silt + clay: <0.063 mm.

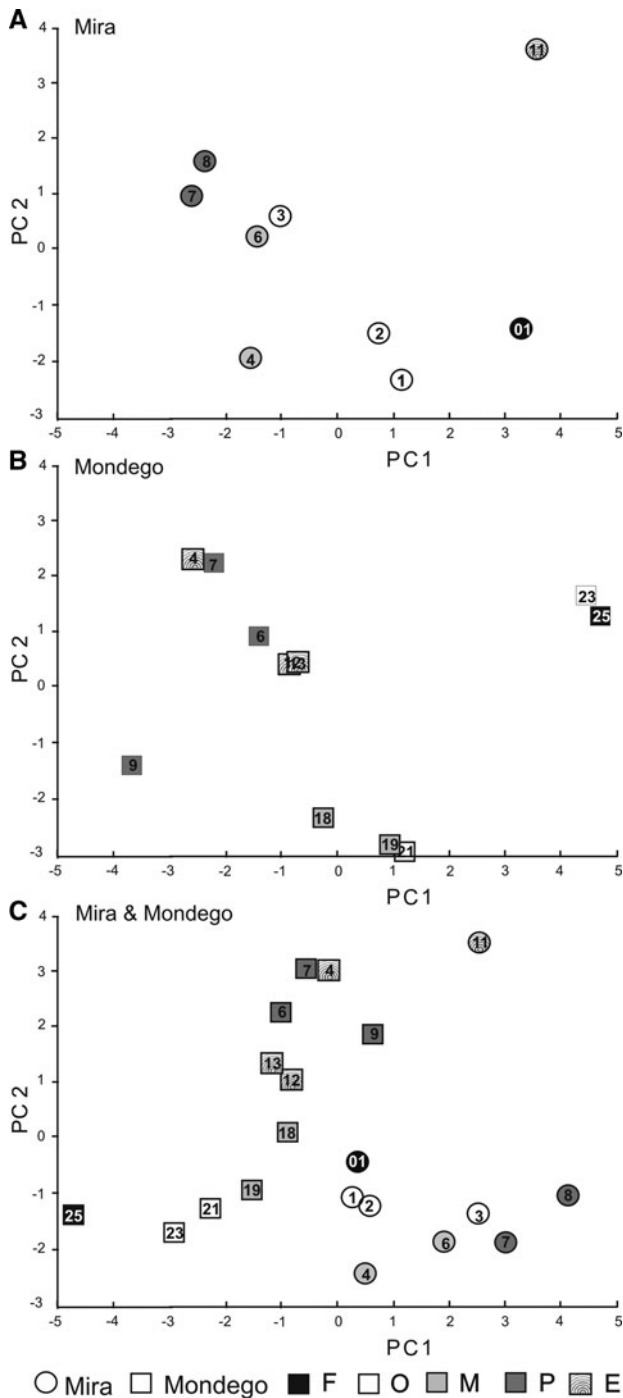


Fig. 2. Principal component analysis (PCA) plot based on the abiotic parameters at each station from: (A) Mira estuary ( $PC_1 = 41.8\%$ ,  $PC_2 = 29.4\%$ ); (B) Mondego estuary ( $PC_1 = 55.4\%$ ,  $PC_2 = 30.2\%$ ); and (C) Mira and Mondego estuaries simultaneously ( $PC_1 = 34.8\%$ ,  $PC_2 = 26.8\%$ ). F, freshwater; O, oligohaline; M, mesohaline; P, polyhaline; E, euhaline.

Group III—included oligohaline and freshwater stations. Similarly to the Mira estuary, the variability obtained in the Mondego analysis was associated with higher proportions of silt + clay in stations located closer to the mouth, and lower salinities and coarser bottom sediments in upstream stations.

Clear differences emerged between the two estuaries when analysing pooled physicochemical data from both estuaries (Figure 2C). The variability along  $PC_1$  was mainly associated

with the estuarine gradients observed in both systems: the stations with higher salinity values and higher proportions of fine particles were located downstream, and the other stations, presenting lower salinities and coarser sediments, were found in upstream areas. Differences between the two systems were essentially associated with the higher OM and silt + clay proportions observed in the Mira estuary, and the higher values of dissolved oxygen and  $PO_4^{3-}$  recorded at Mondego.

## Meiobenthos

The subtidal meiobenthos density and composition followed a clear pattern along the estuarine gradients in both the Mira and the Mondego estuaries.

Meiobenthos mean density was higher in the Mira ( $583.18 \pm 159.23$  ind  $10\text{ cm}^{-2}$ ) than in the Mondego estuary ( $441.65 \pm 88.13$  ind  $10\text{ cm}^{-2}$ ). Significant differences ( $P < 0.05$ ) in density were recorded among the Mira stations, ranging from a minimum of  $14.5 \pm 5.2$  ind  $10\text{ cm}^{-2}$  in Station 01 up to  $2297.4 \pm 426.9$  ind  $10\text{ cm}^{-2}$  in Station 7. There were also significant differences ( $P < 0.05$ ) among the Mondego stations, where a minimum of  $83.7 \pm 20.9$  ind  $10\text{ cm}^{-2}$  was found at Station 25 and a maximum of  $1383.5 \pm 397.1$  ind  $10\text{ cm}^{-2}$  was recorded at Station 4.

Twelve higher taxa were identified in samples from both estuaries: Nematoda, Harpacticoid copepods, Polychaeta, Ostracoda, Nauplii larvae, Bivalvia, Gastropoda, Halacaroida, Turbellaria, Oligochaeta, Amphipoda, and Cladocera. The taxon Ciliophora was found only in the Mondego estuary. Nematodes were the dominant taxon along the estuarine gradients in both systems, representing 95% of the total meiofauna in Mira and 88% in Mondego (Table 3). In the Mira estuary, the maximum nematode density was observed in the polyhaline area (Station 7) ( $2234 \pm 400$  ind  $10\text{ cm}^{-2}$ ), while the minimum occurred in the freshwater Station 01 ( $12.44 \pm 3.91$  ind  $10\text{ cm}^{-2}$ ). In Mondego, on the other hand, nematodes reached the maximum density at the euhaline area in the southern arm (Station 4) ( $1323.10 \pm 389.52$  ind  $10\text{ cm}^{-2}$ ) and the minimum at the freshwater Station 25 ( $38.93 \pm 5.28$  ind  $10\text{ cm}^{-2}$ ). The proportion of nematodes in the total meiofauna density was lowest at the freshwater station of the Mondego estuary (47%), while in the Mira estuary the lowest proportion was found in the euhaline section (70%).

The mean density of other meiofauna groups for both estuaries is also shown in Table 3. Harpacticoid copepods (2%) was the second most abundant taxon in Mira, and Polychaeta (1.8%) the third, with the other taxa representing approximately 1% of the total meiofauna density. In Mondego instead, Polychaeta (8%) were the second most abundant taxon and Harpacticoid copepods (2%) the third. The remaining taxa represented 2% of the total meiofauna density. Differences in meiofauna taxa composition 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 present in the southern one.

The MDS analysis, with stress values of 0.01 and 0.03 (in Mira and Mondego, respectively), generated a good meiofauna data ordination and the 2-dimensional solution was therefore enough to appreciate the overall community structure (Clarke & Warwick, 2001). The ordination based on

**Table 3.** Mean density  $\pm$  SE (number of individuals per 10 cm<sup>2</sup>) of meiofaunal taxa in each station in the Mira and Mondego estuaries.

Estuary	Station	Nematoda	Copepoda	Polychaeta	Ostracoda	Nauplii larvae	Bivalvia	Gastropoda	Halacaroidea	Turbellaria	Oligochaeta	Amphipoda	Cladocera	Ciliophora
Mira	01	12.44 $\pm$ 3.91	–	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	–
	1	267.51 $\pm$ 59.22	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	–	–	–	–
	2	108.97 $\pm$ 26.67	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	–	–
	3	196.07 $\pm$ 79.32	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	–	–	–	–
	4	78.47 $\pm$ 30.66	0.80 $\pm$ 0.80	0.60 $\pm$ 0.60	1.40 $\pm$ 0.40	–	–	–	–	0.20 $\pm$ 0.20	–	–	–	–
	6	202.08 $\pm$ 73.02	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	2234.00 $\pm$ 400.23	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	–	–
	8	1687.34 $\pm$ 200.98	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	–	–	–
11	203.89 $\pm$ 65.25	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	25	38.93 $\pm$ 5.28	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	–	–
	23	100.94 $\pm$ 37.02	1.00 $\pm$ 0.20	34.12 $\pm$ 9.21	–	0.20 $\pm$ 0.20	33.92 $\pm$ 31.25	–	–	–	–	–	–	–
	21	117.40 $\pm$ 12.81	0.60 $\pm$ 0.35	15.85 $\pm$ 4.12	–	–	–	–	–	–	1.40 $\pm$ 0.20	–	–	–
	19	182.62 $\pm$ 51.08	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	–	0.60 $\pm$ 0.60
	18	185.03 $\pm$ 57.73	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	–	–
	13	228.78 $\pm$ 75.23	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
	12	248.04 $\pm$ 37.22	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	–	–	–
	9	1022.08 $\pm$ 266.11	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	–	–	–	–	–
	7	725.27 $\pm$ 308.19	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	–	–	–
	6	103.55 $\pm$ 36.01	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	–	–
4	1323.10 $\pm$ 389.52	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	–	

(–), absence of taxon in the station.

the meiofauna taxa abundance and composition from the Mira estuary showed clearly the spatial heterogeneity along the estuarine gradient (Figure 3A). The ANOSIM analysis showed significant differences between salinity stretches (Global  $R = 0.709$ ,  $P = 0.1\%$ ). The freshwater assemblage was significantly different from all other assemblages ( $R_{F/O} = 0.962$ ,  $P = 0.5\%$ ;  $R_{F/M} = 0.772$ ,  $P = 1.2\%$ ;  $R_{F/P} = 1$ ,  $P = 1.2\%$ ). The oligohaline assemblages were significantly different from the polyhaline and euhaline assemblages ( $R_{O/P} = 0.927$ ,  $P = 0.1\%$ ;  $R_{O/E} = 0.613$ ,  $P = 0.5\%$ ) and closely related with the mesohaline group ( $R_{O/M} = 0.079$ ,  $P = 19.7\%$ ). The polyhaline assemblages were significantly different from the mesohaline and euhaline communities ( $R_{P/M} = 0.869$ ,  $P = 0.2\%$ ;  $R_{P/E} = 1$ ,  $P = 1.2\%$ ). The meiofauna assemblage structure in the euhaline area was significantly similar to the one in the mesohaline stretch ( $R_{E/M} = 0.185$ ,  $P = 11.9\%$ ). The SIMPER analysis showed that dissimilarities between salinity stretches were mainly caused by the taxa Nematoda,

Harpacticoid copepods and Polychaeta. The higher dissimilarities were observed between freshwater and polyhaline stretches (98.6%) due to higher density of nematodes in this last section. Also, the high dissimilarity between freshwater and euhaline stretches (89.11%) was caused by the higher density of Nematoda, Polychaeta and Harpacticoid copepods in the euhaline section. The BIOENV analysis showed that salinity, gravel content, DO, water nitrites and phosphate concentrations were the main factors related to meiofaunal distribution (Table 4).

The MDS analysis of meiofauna taxa density data from the Mondego estuary also showed spatial heterogeneity along the estuarine gradient (Figure 3B). This MDS ordination discriminated the stations located in the southern arm and Pranto River (4, 7 and 9) from the stations in the northern arm (13 and 12). Moreover, ANOSIM analysis detected significant differences between salinity stretches (Global  $R = 0.253$ ,  $P = 0.2\%$ ). SIMPER analysis showed a dissimilarity maximum (74.52%) between assemblages from the freshwater and euhaline sections, mainly due to the higher nematodes density in the euhaline area and higher Polychaeta density in the freshwater area. The minimum dissimilarity was observed between meiofauna communities from oligohaline and mesohaline stretches (36.86%). The BIOENV analysis showed that the water ammonium was the physicochemical variable accounting for 68% of the variability within the Mondego estuary's meiofauna community.

The MDS analysis was not able to discriminate between the Mondego and Mira assemblages when using pooled data from the assemblages in both estuaries (Figure 3C). However, ANOSIM analysis showed that there were significant differences between estuaries (Global  $R = 0.14$ ,  $P = 0.2\%$ ) and stretches (Global  $R = 0.339$ ,  $P = 0.1\%$ ). Furthermore, it was possible to discriminate three groups of stations: (i) freshwater stations—where total meiofauna, nematodes and Harpacticoid copepods density reached minimum values (15 to 84 ind  $10\text{ cm}^{-2}$ ); (ii) oligohaline and mesohaline stations—where total meiobenthic density and number of taxa were lower (81 to 292 ind  $10\text{ cm}^{-2}$ ); and (iii) polyhaline and euhaline stations—where total meiofauna reached the highest density and the highest number of taxa (833 to 2297 ind  $10\text{ cm}^{-2}$ ). However, there were deviations from this general trend. For instance, the assemblages of the Mira euhaline station were similar to this estuary's mesohaline communities, and there were differences in the euhaline assemblages between the north and south arms of the Mondego estuary.

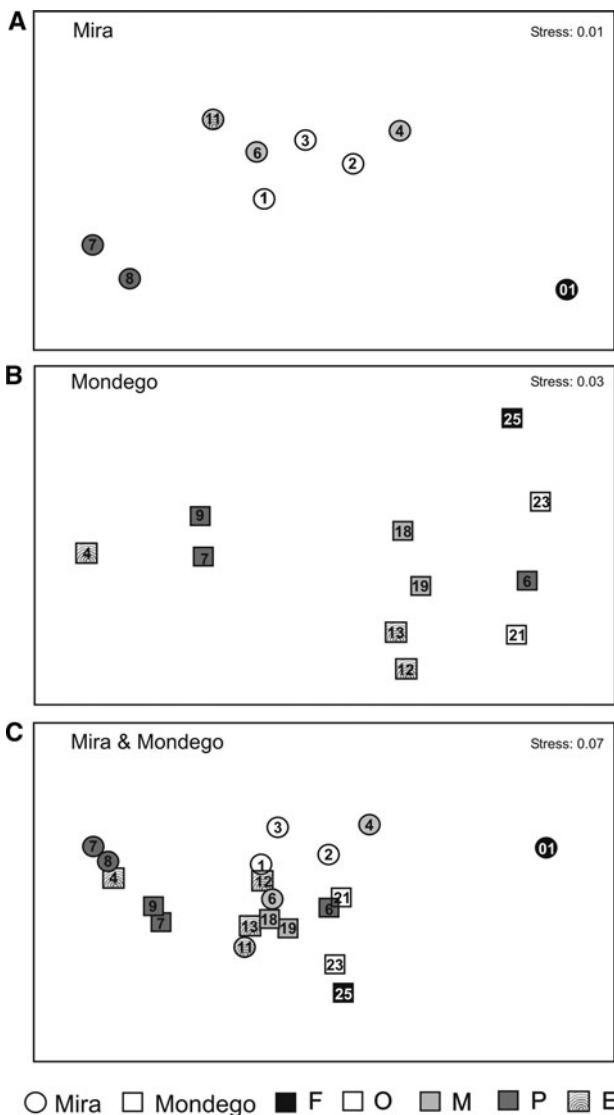


Fig. 3. Non-metric multidimensional scaling (MDS) plot based on the mean density of the three meiofaunal taxa replicates, from sampling stations: (A) Mira estuary; (B) Mondego estuary; and (C) Mira and Mondego estuaries simultaneously.

Table 4. BIOENV results carried out to meiofauna and environmental factors data.

Estuary	No. variables	Correlation	Selection
Mira	5	0.545	Salinity, gravel, DO, $\text{PO}_4^{3-}$ , $\text{NO}_3^-$
	5	0.534	Salinity, DO, pH, $\text{PO}_4^{3-}$ , $\text{NO}_3^-$
	5	0.523	Salinity, coarse sand, DO, pH, $\text{PO}_4^{3-}$
Mondego	1	0.682	$\text{NH}_4^+$
	2	0.666	$\text{PO}_4^{3-}$ , $\text{NH}_4^+$
	3	0.659	pH, $\text{PO}_4^{3-}$ , $\text{NH}_4^+$

DO, dissolved oxygen;  $\text{PO}_4^{3-}$ , phosphate;  $\text{NO}_3^-$ , nitrate;  $\text{NO}_2^-$ , nitrite;  $\text{NH}_4^+$ , ammonium; gravel: >2 mm; coarse sand: 0.5–2.0 mm.



## DISCUSSION AND CONCLUSIONS

The estuarine gradients of salinity, particles size and water nutrients were clearly detected at both estuaries.

In Mira, the typical transitional water gradients were very clear and mostly explained by the system's morphology—a single river channel and an almost complete absence of irregularities in its terminal section—allowing the tidal influence to extend about 40 km inland (Paula *et al.*, 2006). Therefore, the mouth areas, with higher marine influence, are different from those at the inner sections, with essentially polyhaline and mesohaline conditions, and both of these differ from those at the head, which are under oligohaline conditions and fresh tide influence (Elliot & McLusky, 2002).

In Mondego, due to the distinct hydrological regimes of the northern and southern arms, the estuarine gradients are evident, although more complex. The northern arm is deeper and has been heavily modified, particularly 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 tide. On the contrary, the south arm is much shallower, constituting almost a kind of coastal lagoon in which the water circulation depends mostly on the tides and on the freshwater input from the discharge, irregular and controlled by a sluice, of a small tributary, the Pranto River.

A typical gradient of estuarine sediments was observed in Mira, with fractions of silt and clay increasing from the upstream sections towards the mouth of the estuary. On the contrary, the gradient of sediments was much less clear in the Mondego estuary. In general, in Mondego, subtidal bottoms had larger fine sand fractions and much smaller silt and clay fractions than in Mira. Moreover, sediments distribution was very distinct between the two arms of the Mondego estuary (Teixeira *et al.*, 2008).

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

The higher organic matter content and the higher proportions of silt and clay could explain the higher meiofauna density registered in the Mira estuary. The nematodes taxon dominated the communities in both estuaries and the density was in agreement with the range observed in subtidal environments of the northern European estuaries, values which are generally lower than those from intertidal systems (Smol *et al.*, 1994; Soetaert *et al.*, 1994). In Mondego, the

proportion of nematodes decreased in the freshwater section, thus presenting a similar pattern to that observed in several other estuaries (Smol *et al.*, 1994; Soetaert *et al.*, 1994, 1995; Udalov *et al.*, 2005), while in the Mira estuary the proportion of nematodes remained constant and no visible trend was obtained. Harpacticoid copepods came second in relative abundance in the Mira estuary, while in Mondego the same position belonged to Polychaeta, followed by Harpacticoid copepods. Copepoda are typically ranked second in order of abundance in estuarine sediments and only occasionally is that position occupied by another taxon (Coull, 1999).

Comparable values of meiobenthos density to those observed in the present study (15–2297 ind 10 cm<sup>-2</sup> in the Mira and 84–1384 ind cm<sup>-2</sup> in the Mondego estuary) as well as a similar pattern of increasing density seawards, were reported in subtidal sediments in the Westerschelde estuary (67–1666 ind 10 cm<sup>-2</sup>) (Soetaert *et al.*, 1994) and in intertidal sediments in the Chernaya River (167–2356 ind 10 cm<sup>-2</sup>) (Udalov *et al.*, 2005).

In summary, there was significant spatial heterogeneity in the subtidal meiobenthos assemblages' abundance and composition along the estuarine environmental gradients of both estuaries. Three main areas were identified across the estuaries: (i) the freshwater sections—total meiofauna, nematodes and Harpacticoid copepods density reached minimum values; (ii) the oligohaline and mesohaline sections—total meiobenthic density was low and diversity was poor; and (iii) the polyhaline and euhaline sections—total meiofauna reached the highest density values and the highest diversity. This tendency was also observed in a number of other studies regarding intertidal and subtidal meiofauna communities (Heip *et al.*, 1985; Li & Vincx, 1993; Soetaert *et al.*, 1994, 1995; Yamamuro, 2000).

It is widely accepted that one of the main factors influencing species distribution in estuaries is salinity (Bulger *et al.*, 1993; Atrill, 2002; McLusky & Elliot, 2004). Several studies identified salinity as an important independent factor determining meiobenthic communities' structure and describing total meiobenthic density and diversity changes (Coull, 1985; Vincx *et al.*, 1990; Soetaert *et al.*, 1995; Santos *et al.*, 1996). Some freshwater species, and even terrestrial species, can invade water less than 5 in the salinity scale. At the same time, the downstream areas of estuaries are invaded by marine species that have to adapt to reduced salinity in variable degrees and that eventually vanish with decreasing salinity. Estuarine meiofauna tends to decrease in abundance and number of species as one moves from the sea to freshwater (Bouwman, 1983; Heip *et al.*, 1985; Coull, 1999).

Along with salinity, sediment composition is recognized as another very important factor influencing meiobenthic taxa composition and abundance patterns (Coull, 1985; Vincx *et al.*, 1990; Steyaert *et al.*, 2003), and driving important deviations from the general trend shaped by salinity. For instance, the meiobenthos density was lower in the intermediate salinity zones of the Tamar estuary intertidal sediments (Austen & Warwick, 1989) and in subtidal sediments of estuarine lagoons (Yamamuro, 2000). In the Westerschelde estuary, the meiofauna distribution (namely nematode species) is greatly influenced by the sedimentary and geomorphological heterogeneity and, together with salinity and related gradients, these factors explain many of the differences observed between communities (Soetaert *et al.*, 1994). These studies

considered the salinity gradient an important driver controlling assemblages' spatial distribution; however, other factors such as sediment type can overlap the salinity effects. Indeed, the results observed in the Mira estuary euhaline area can most probably be attributed to grain size composition.

In the present study we confirmed a role of salinity in shaping the distribution of meiofauna communities, and we also demonstrated that other environmental factors, such as granulometry, nutrients concentration and sediment organic matter content, may interact and prevail over salinity effects. In fact, meiofauna density deviations from the general trend were observed in the euhaline stretch of the Mira estuary. This area contained a meiofauna density similar to that of the mesohaline area most likely due to the influence of the sediment grain size (higher proportion of sand and a concomitant reduction of the silt + clay fraction in the euhaline area). Another example could be found when comparing the Mondego northern and southern arms' meiofauna assemblages. The different types of environmental impacts in the northern and southern Mondego branches were extensively described in previous studies (Marques *et al.*, 2007; Patrício *et al.*, 2007; Teixeira *et al.*, 2008). The northern arm has mainly experienced physical disturbance (stonewalls construction and regular dredging) and no eutrophication symptoms have been detected so far. On the contrary, in the southern branch, it was possible to see clear eutrophication symptoms during the 1990s (resulting from high nutrient loading together with hydrodynamic confinement). Nevertheless, since 2000–2001, and despite the prevalent high nutrient loading, no more opportunistic macroalgae blooms were observed in this area and, as a result, the *Zostera noltii* meadows area is slowly recovering (Marques *et al.*, 2007). During the spring of 2006, the full re-establishment of the communication between the two arms was undertaken (Marques *et al.*, 2007). This intervention appeared as a suitable way of improving the south arm water circulation and consequently its ecological quality. Sampling for the present study was done afterwards (summer 2006). At the same time, this branch has sediments with higher silt and clay proportion, higher organic matter content and a minor freshwater discharge. Physical disturbance is the major anthropogenic driver acting in the northern branch and high nutrient concentrations (especially ammonium and phosphates) is a major pollution problem in both arms. Our results suggest that the natural stressors related with sedimentary and geomorphological characteristics, together with salinity and related estuarine gradients are the major factors shaping meiofauna distribution patterns at both subsystems in the Mondego estuary.

*A priori*, we would expect 'natural stressors' to be the major meiofauna density and diversity regulators in the Mira estuary. In contrast, we expected the 'higher anthropogenic stress' in the Mondego estuary to cause significant changes in meiobenthic distribution along the estuarine gradient. However, considering both estuaries, the meiofauna spatial distribution followed a similar pattern, reflecting once more the natural variability along the estuarine gradients and supporting other studies claiming that meiofauna variability within estuaries (in the order of kilometres), due to salinity changes or grain size differences, is more important than variability at the scale hundreds of kilometres among estuaries (Soetaert *et al.*, 1995; Li *et al.*, 1997).

From a management perspective, meiofauna attributes, such as density, composition or taxa number, could provide a useful tool to evaluate estuarine water quality, leading to lower sampling effort and minor time consumption (both in the field and laboratory). However, the results from higher taxa resolution of these meiofauna assemblages revealed an obvious constraint in applying such ecological quality evaluation tools to detect anthropogenic-driven changes. Our results, challenge Warwick's (1988) point in suggesting that anthropogenic effects modify community composition at a higher taxonomic level than natural environmental variables. Moreover, our results are in good agreement with the 'Estuarine Quality Paradox' (Dauvin, 2007), which is based on the central thesis that estuaries are naturally stressed because of the high degree of variability in their physicochemical characteristics; therefore the structural features of the estuarine communities under natural stress resemble those of anthropogenic stressed areas, making it very difficult to distinguish communities from natural or human induced areas (Elliot & Quintino, 2007).

## ACKNOWLEDGEMENTS

The present study was carried out within the scope of the research projects EFICAS (POCI/MAR/61324/2004) and RECONNECT (PTDC/MAR/64627/2006). It was also supported by FCT (Portuguese National Board of Scientific Research) through two grants (SFRH/BPD/26604/2006, SFRH/BPD/41701/2007). A special acknowledgement is due to the CIEMAR laboratory (Évora University) for the logistic support. An anonymous referee is thanked for the comments that allowed the improvement of the manuscript.

## REFERENCES

- Anon (1959) Symposium on the Classification of Brackish Waters. Venice, 8–14 April 1958. *Archivio di Oceanografia e Limnologia*, 11 (supplement).
- Atrill M. (2002) A testable linear model for diversity trends in estuaries. *Journal of Animal Ecology* 71, 262–269.
- Austen M.C. and Warwick R.M. (1989) Comparison of univariate and multivariate aspects of estuarine meiobenthic community structure. *Estuarine, Coastal and Shelf Science* 29, 23–42.
- Bouman L.A. (1983) A survey of Nematoda from the Ems estuary: species assemblages and associations. *Zoologische Jahrbucher, Systematik, Ökologie und Geographie der Tiere* 110, 345–376.
- Brown A.C. and McLachlan A. (1990) *Ecology of sandy shores*. Amsterdam: Elsevier.
- Bulger A.J., Hayden B.P., Monaco M.E., Nelson D.M. and McCormick-Ray M.G. (1993) Biologically-based estuarine salinity zones derived from a multivariate analysis. *Estuaries* 16, 311–322.
- Clarke K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18, 117–143.
- Clarke K.R. and Ainsworth M. (1993) A method for linking multivariate community structure to environmental variables. *Marine Ecology Progress Series* 92, 205–219.
- Clarke K.R. and Warwick R.M. (2001) *Change in marine communities: an approach to statistical analysis and interpretation*. 2nd edition. Plymouth: Plymouth Marine Laboratory.

- Costa M.J., Catarino F. and Bettencourt A. (2001) The role of salt marshes in the Mira estuary (Portugal). *Wetlands Ecology and Management* 9, 121–134.
- Coull B.C. (1985) Long-term variability of estuarine meiobenthos: an 11 year study. *Marine Ecology Progress Series* 24, 205–218.
- Coull B.C. (1999) Role of meiofauna in estuarine soft-bottom habitats. *Australian Journal of Ecology* 24, 327–343.
- Coull B.C. and Chandler T. (1992) Pollution and meiofauna field, laboratory and mesocosm studies. *Oceanography and Marine Biology: an Annual Review* 30, 191–271.
- Dauvin J.-C. (2007) Paradox of estuarine quality: benthic indicators and indices, consensus or debate for the future. *Marine Pollution Bulletin* 55, 271–281.
- Derycke S., Hendrickx F., Backeljau T., D'Hondt S., Camphijn L., Vincx M. and Moens T. (2007) Effects of sublethal abiotic stressors on population growth and genetic diversity of *Pellioiditis marina* (Nematoda) from the Westerschelde estuary. *Aquatic Toxicology* 82, 110–119.
- Elliot M. and McLusky D.S. (2002) The need for definitions in understanding estuaries. *Estuarine, Coastal and Shelf Science* 55, 815–827.
- Elliot M. and Quintino V. (2007) The estuarine quality paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin* 54, 640–645.
- Essink K. and Keidel H. (1998) Change in estuarine nematode communities following a decrease of organic pollution. *Aquatic Ecology* 32, 195–202.
- Flindt M.R., Kamp-Nielsen L., Marques J.C., Pardal M.A., Bocci M., Bendoricchio G., Salomonsen J., Nielsen S.N. and Jørgensen S.E. (1997) Description of three shallow estuaries: Mondego river (Portugal), Roskilde Fjord (Denmark) and the lagoon of Venice (Italy). *Ecological Modelling* 102, 17–31.
- Forster S.J. (1998) Osmotic stress tolerance and osmoregulation of intertidal and subtidal nematodes. *Journal of Experimental Marine Biology and Ecology* 224, 109–125.
- Giere O. (1993) *Meiobenthology*. Berlin: Springer-Verlag.
- Heip C.H., Vincx M. and Vranken G. (1985) The ecology of marine Nematoda. *Oceanography and Marine Biology: an Annual Review* 23, 399–489.
- Higgins P.R. and Thiel H. (1988) *Introduction to the study of meiofauna*. Washington, DC: Smithsonian Institution Press.
- Li J. and Vincx M. (1993) The temporal variation of intertidal nematodes in the Westerschelde. I. The importance of an estuarine gradient. *Netherlands Journal of Aquatic Ecology* 27, 319–326.
- Li J., Vincx M., Herman P.M.J. Heip C.H. (1997) Monitoring meiobenthos using cm-, m- and km-scales in the Southern Bight of the North Sea. *Marine Environmental Research* 34, 265–278.
- Lillebø A.I., Teixeira H., Pardal M.A. and Marques J.C. (2007) Applying quality status criteria to a temperate estuary before and after the mitigation measures to reduce eutrophication symptoms. *Estuarine, Coastal and Shelf Science* 72, 177–187.
- Limnologisk Metodik (1992) *Ferskvandsbiologisk Laboratorium*. In Universitet Kobenhavns (ed.) Kobenhavn: Akademisk Forlag.
- Marques J.C., Maranhão P. and Pardal M.A. (1993) Human impact assessment on the subtidal macrobenthic community structure in the Mondego Estuary (Western Portugal). *Estuarine, Coastal and Shelf Science* 37, 403–419.
- Marques J.C., Neto J.M., Patrício J., Pinto R., Teixeira H. and Veríssimo H. (2007) *Monitoring the Mondego estuary. Anthropogenic changes and their impact on ecological quality. Preliminary results from the first assessment of the effects of reopening the communication between the North and South arms on the eutrophication state of the system*. Final Report, January 2007. IMAR/INAG, 87 pp.
- McLusky D.S. and Elliot M. (2004) *The estuarine ecosystem—ecology, threats and management*. Oxford: Oxford University Press.
- Moens T. and Vincx M. (2000) Temperature and salinity constraints on the life cycle of two brackish-water nematode species. *Journal of Experimental Marine Biology and Ecology* 243, 115–135.
- Neto J.M., Flindt M.R., Marques J.C. and Pardal M.A. (2008) Modelling nutrient mass balance in a meso-tidal estuary: implications for management. *Estuarine, Coastal and Shelf Science* 76, 175–185.
- Parsons T.R., Takahashi M. and Hargrave B. (1990) *Biological oceanographic processes*. 3rd edition. Oxford: Pergamon Press.
- Patrício J., Neto J.M., Teixeira H. and Marques J.C. (2007) Opportunistic macroalgae metrics for transitional waters. Testing tools to assess ecological quality status in Portugal. *Marine Pollution Bulletin* 54, 1887–1896.
- Paula J., Silva I.C., Francisco S.M. and Flores A.V. (2006) The use of artificial benthic collectors for assessment of spatial patterns of settlement of megalopae of *Carcinus maenas* (L.) (Brachyura: Portunidae) in the lower Mira Estuary, Portugal. *Hydrobiologia* 557, 69–77.
- Phillips F.E. and Fleeger J.W. (1985) Meiofauna meso-scale variability in two estuarine habitats. *Estuarine, Coastal and Shelf Science* 21, 745–756.
- Sandulli R. and Pickney J. (1999) Patch sizes and spatial patterns of meiobenthic copepods and benthic microalgae in sandy sediments: a microscale approach. *Journal of Sea Research* 41, 179–187.
- Santos P.J.P., Castel J. and Souza-Santos L.P. (1996) Seasonal variability of meiofaunal abundance in the oligo-mesohaline area of the Gironde Estuary, France. *Estuarine, Coastal and Shelf Science* 43, 549–563.
- Schratzberger M. and Warwick R.M. (1998) Effects of the intensity and frequency of organic enrichment on two estuarine nematode communities. *Marine Ecology Progress Series* 164, 83–94.
- Schratzberger M., Bolam S., Whomersley P., Warr K. and Rees H. (2004) Development of a meiobenthic nematode community following the intertidal placement of various types of sediment. *Journal of Experimental Marine Biology and Ecology* 303, 79–96.
- Smol N., Willems K.A., Govaere J.C. and Sandee A.J.J. (1994) Composition, distribution and biomass of meiobenthos in the Oosterschelde estuary (SW Netherlands). *Hydrobiologia* 282/283, 197–217.
- Soetaert K., Vincx M., Wittoeck J., Tulkens M. and Van Gansbeke D. (1994) Spatial patterns of Westerschelde meiobenthos. *Estuarine, Coastal and Shelf Science* 39, 367–388.
- Soetaert K., Vincx M., Wittoeck J. and Tulkens M. (1995) Meiobenthic distribution and nematode community structure in five European estuaries. *Hydrobiologia* 311, 185–206.
- Somerfield P.J. and Clarke K.R. (1995) Taxonomic levels, in marine community studies revisited. *Marine Ecology Progress Series* 127, 113–119.
- Steyaert M., Vanaberbeke J., Vanreusel A., Barranguet C., Lucas C. and Vincx M. (2003) The importance of fine-scale, vertical profiles in characterizing nematode community structure. *Estuarine, Coastal and Shelf Science* 58, 353–366.
- Strickland J.D.M. and Parsons T.R. (1972) *A practical handbook of seawater analysis*. (Bulletin 167, second edition). Ottawa: Fisheries Research Board of Canada, 311 pp.

- Teixeira H., Salas F., Borja A., Neto J.M. and Marques J.C.** (2008) A benthic perspective in assessing the ecological status of estuaries: the case of the Mondego estuary (Portugal). *Ecological Indicators* 8, 404–416.
- Udalov A.A., Mokievskii V.O. and Chertoprud E.S.** (2005) Influence of the salinity gradient on the distribution of meiobenthos in the Chernaya River Estuary (White Sea). *Oceanology* 45, 680–688.
- Vincx M.** (1996) Meiofauna in marine and freshwater sediments. In Hall G.S. (ed.) *Methods for the examination of organismal diversity in soils and sediments*. Wallingford, UK: Cab International, pp. 187–195.
- Vincx M., Meire P. and Heip C.H.** (1990) The distribution of the Nematoda communities in Southern Bight of the North Sea. *Cahiers de Biologie Marine* 31, 439–462.
- Warwick R.M.** (1981) The nematode/copepod ratio and its use in pollution ecology. *Marine Pollution Bulletin* 12, 329–333.
- Warwick R.W.** (1988) The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. *Marine Pollution Bulletin* 19, 259–268.
- Warwick R.W.** (1993) Environmental impact studies on marine communities: pragmatic considerations. *Australian Journal of Ecology* 18, 63–80.
- and
- Yamamuro M.** (2000) Abundance and size distribution of sublittoral meiobenthos along estuarine salinity gradients. *Journal of Marine Systems* 26, 135–143.

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