

Benthic meiofauna as indicator of ecological changes in estuarine ecosystems: The use of nematodes in ecological quality assessment

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ARTICLE INFO

Article history:

Received 25 April 2012

Received in revised form 12 July 2012

Accepted 21 July 2012

Keywords:

Meiobenthos
Free-living nematodes
Indicators
Biodiversity
Estuaries

ABSTRACT

Estuarine meiofauna communities have been only recently considered to be good indicators of ecological quality, exhibiting several advantages over macrofauna, such as their small size, high abundance, rapid generation times and absence of a planktonic phase. In estuaries we must account not only for a great natural variability along the estuarine gradient (e.g. sediment type and dynamics, oxygen availability, temperature and flow speed) but also for the existence of anthropogenic pressures (e.g. high local population density, presence of harbors and dredging activities).

Spatial and temporal biodiversity patterns of meiofauna and free-living marine nematodes were studied in the Mondego estuary (Portugal). Both taxonomic and functional approaches were applied to nematode communities in order to describe the community structure and to relate it with the environmental parameters along the estuary. At all sampling events, nematode assemblages reflected the estuarine gradient, and salinity and grain size composition were confirmed to be the main abiotic factors controlling the distribution of the assemblages.

Moreover, the low temporal variability may indicate that natural variability is superimposed by the anthropogenic pressures present in some areas of the estuary. The characterization of both meiofauna and nematode assemblages highlighted the usefulness of the integration of both taxonomic and functional attributes, which must be taken into consideration when assessing the ecological status of estuaries.

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1. Introduction

Meiofauna features are a good indicator of environmental conditions and changes in their density, diversity, structure and functioning may indicate alterations in the system. Although not being included in the biological compartment that needs to be monitored in the scope of the Water Framework Directive (WFD, Directive 2000/60/EC), meiofauna gives valuable information regarding ecosystems health. According to Sheppard (2006), marine scientists need to increase awareness of and emphasize the importance of the many species that have no appeal, which are not attractive and, for the most part, are not seen, like meiofauna.

Despite these difficulties, meiofauna communities are reasonably well characterized around the world, with studies ranging from the deep sea floor to alpine lakes, as well as from tropical

reefs to polar sea ice (Giere, 2009). In Europe, studies on meiobenthic communities mostly encompass the more northerly estuarine ecosystems (e.g. Warwick and Gee, 1984; Li and Vincx, 1993; Smol et al., 1994; Soetaert et al., 1995; Ferrero et al., 2008). In southern Europe there is a serious gap in knowledge. Particularly in the Iberian Peninsula, there is a lack of information on both spatial and temporal distribution of meiofauna and free living nematodes in estuarine environments, being essential to describe those biodiversity patterns.

Meiobenthic communities provide information of great interest not only due to their important role in marine benthic food chains (Heip et al., 1985; Moens et al., 2005) but also due to their ecological characteristics (small size, high abundance, rapid generation times and absence of a planktonic phase), giving meiofauna several advantages over the commonly used macrofauna communities as monitoring organisms (Kennedy and Jacoby, 1999; Schratzberger et al., 2000; Austen and Widdicombe, 2006). In fact, nematodes have been pointed out as potential indicators of anthropogenic disturbance in aquatic ecosystems (e.g. Coull and Chandler, 1992; Schratzberger et al., 2004; Steyaert et al., 2007; Moreno et al., 2008). The inclusion of information regarding their functional traits

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(e.g. trophic structure, life strategy) can provide critical information on the functioning of ecosystems (Norling et al., 2007; Danovaro et al., 2008).

Estuaries are naturally stressed systems with a high degree of variability in their physical–chemical characteristics. The natural gradient of salinity, linked with other gradients (e.g. bed sediment type and dynamics, oxygen availability, temperature and current speed), are well documented as important factors in determining temporal and spatial variations of meiofauna communities (Bouwman, 1983; Heip et al., 1985; Austen and Warwick, 1989; Soetaert et al., 1995; Li et al., 1997; Forster, 1998; Moens and Vincx, 2000; Steyaert et al., 2003; Derycke et al., 2007; Alves et al., 2009; Adão et al., 2009) but studies encompassing the entire salinity range from marine to freshwater conditions are few (e.g. Portugal: Alves et al., 2009; Adão et al., 2009; Patrício et al., 2012; United Kingdom: Ferrero et al., 2008; The Netherlands: Soetaert et al., 1994, Australia: Hourston et al., 2011). Moreover, most studies cover a small temporal range, providing only limited information on the behavior of assemblages over longer time scales.

The present study compares the characteristics of meiofauna and free living nematodes assemblages in the subtidal sediments of different locations from Euhaline to Oligohaline areas of the Mondego estuary. Furthermore, the temporal (seasonal) variability between the assemblages of different locations is assessed and the use of nematodes as biological indicators of environmental quality is considered.

This study aimed to investigate changes in patterns of meiofauna and nematode assemblage composition and nematode diversity, trophic composition and life strategies between different estuarine locations and sampling occasions

The following null hypotheses were tested: (a) there would be no differences in meiofauna taxon and nematode assemblage composition and trophic composition along the estuary; and (b) there would be no differences in the meiofaunal taxon and nematode assemblage composition and trophic composition at different seasonal sampling events.

2. Materials and methods

2.1. Study area

The Mondego estuary (Fig. 1), located on the Atlantic coast of Portugal (40°08'N, 8°50'W), is a polyhaline system influenced by a warm-temperate climate. The estuary is 21 km long (based on the extent of tidal influence) with an area of about 8.6 km² and, in its terminal part (at a distance of 7 km from the sea) it divides into two arms, northern and southern, separated by an alluvial island (Murraceira island), which rejoin near the estuary's mouth. The two arms have very different hydrological characteristics. The northern arm is deeper (5–10 m during high tide), receives most of the

system's freshwater input, being influenced by seasonal fluctuation in water flow (Flindt et al., 1997), and forms the main navigation channel on which the Figueira da Foz harbor is located. The southern arm is shallower (2–4 m during high tide), has large areas of intertidal mudflats (almost 75% of the area) exposed during low tide and, until the Spring of 2006, was almost silted up in the upper zones. In May 2006, the communication between both arms was re-established in order to improve the water quality in the terminal part of the estuary by reducing the residence time in the southern arm (Neto et al., 2010).

The Mondego estuary supports not only the Figueira da Foz harbor (regular dredging is carried out to ensure shipping conditions) but also numerous industries and receives agricultural run-off from rice and corn fields in the Lower River valley (Marques et al., 2003).

2.2. Sampling strategy

The subtidal soft-bottom meiobenthic assemblages were sampled along the salinity gradient of the Mondego estuary on six sampling occasions: August 2006 (summer, Su06), November 2006 (autumn, Au06), March 2007 (winter, Wi07), June 2007 (spring, Sp07), September 2009 (summer, Su09) and December 2009 (autumn, Au09).

Eleven sampling stations were selected following the division of the estuary proposed by Teixeira et al. (2008) (Fig. 1). The estuary was thus divided in five different areas: Euhaline (station 4; salinity 30–34); Polyhaline of the South Arm (st 6, 7 and 9; salinity 18–30), Polyhaline of the North Arm (st 12 and 13; salinity 18–30), Mesohaline (18 and 19; salinity 5–18) and Oligohaline (st 21, 23 and 25; salinity 0.5–5).

2.2.1. Environmental data

At each sampling station, bottom water parameters were measured *in situ* with a YSI Data Sonde Survey 4: salinity (Practical Salinity Scale) (in Autumn 2009 – no salinity data was recorded), temperature (°C), pH, and dissolved oxygen (DO) (mg L⁻¹). Water samples were collected for determination of nutrients and chlorophyll *a* (mg m⁻³) in laboratory: nitrate (NO₃⁻-N) and nitrite (NO₂⁻-N) concentrations (μmol L⁻¹) were analyzed according to standard methods described in Strickland and Parsons (1972) and ammonium (NH₄⁺-N) and phosphate (PO₄³⁻-P) concentrations (μmol L⁻¹) were analyzed following the Limnological Metodik (1992). Chlorophyll *a* (Chl *a*) determinations were performed according to Parsons et al. (1985). Sediment samples were taken at each station to determine the organic matter content and grain size. Sediment organic matter (OM) content was defined as the difference between the weight of each sample after oven-drying at 60 °C for 72 h followed by combustion at 450 °C for 8 h, and was expressed as the percentage of the total weight. Grain size was analyzed by dry mechanical separation through a column of sieves of

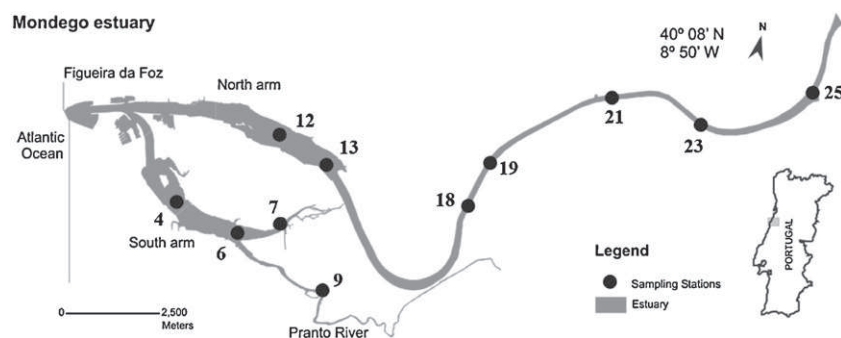


Fig. 1. Mondego estuary (Portugal): station location (black circles). Areas: Euhaline (station 4), Polyhaline of the South Arm (stations 6, 7 and 9), Polyhaline of the North Arm (stations 12 and 13), Mesohaline (stations 18 and 19) and Oligohaline (stations 21, 23 and 25).

different mesh sizes, corresponding to the five classes described by Brown and McLachlan (1990): (a) gravel (>2 mm), (b) coarse sand (0.500–2.000 mm), (c) mean sand (0.250–0.500 mm), (d) fine sand (0.063–0.250 mm), and (e) silt and clay (<0.063 mm). The relative content of the different grain-size fractions was expressed as a percentage of the total sample weight.

2.2.2. Biological data

Three replicate samples 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 and were sieved through 1 mm and 38 μm mesh size sieves (material retained on the smaller mesh was collected). Mei fauna was extracted from the sediment fraction using Ludox HS-40 colloidal silica at a specific gravity of 1.18 g cm^{-3} (Vincx, 1996). All meiobenthic organisms were identified to major taxa level under a stereomicroscope using Higgins and Thiel (1988) and Giere (2009) and the density (individuals per 10 cm^2) of each taxon was quantified.

From each replicate, a random set of 120 nematodes, or the total number of individuals in samples with less than 120 nematodes, were picked, cleared in glycerol–ethanol solution, transferred to anhydrous glycerol by evaporation and mounted on slides for identification (Vincx, 1996). All nematodes were identified to genus level using a microscope fitted with a 100 \times oil immersion objective and based on the pictorial keys of Platt and Warwick (1983, 1988), Warwick et al. (1998), the online information system NeMys (Steyaert et al., 2005) and on Abebe et al. (2006).

2.3. Data analysis

Univariate and multivariate analyses to detect spatial and temporal changes in the community structure were performed according to the procedures described by Clarke (1993), using the PRIMER v6 software package (Clarke and Warwick, 2001) with the PERMANOVA add-on package (Anderson et al., 2008).

2.3.1. Environmental variables

A Principal Component Analysis (PCA) of the environmental variables was performed to find patterns in multi-dimensional data by reducing the number of dimensions, with minimal loss of information. Prior to the calculation of the environmental parameter resemblance matrix based on Euclidean distance, the environmental variables (temperature, salinity, dissolved oxygen, pH, ammonium, nitrate, nitrite, phosphate, silicates, organic matter and each of the five granulometric classes) were square-root transformed (except dissolved oxygen and pH data) and followed normalization.

2.3.2. Mei fauna assemblages

Total mei fauna density and density of individual major mei fauna taxa (individuals per 10 cm^2) were calculated, for each area and sampling occasion.

In order to test the hypothesis that the composition of mei fauna changes spatially and seasonally, a two-way PERMANOVA analysis was carried out with the following crossed factor design: “area” and “sampling occasion” as fixed factors, with five (Euhaline, Polyhaline North Arm, Polyhaline South Arm, Mesohaline and Oligohaline) and six levels (Su06, Au06, Wi07, Sp07, Su09 and Au09), respectively. Mei fauna taxa density data were square root transformed in order to scale down densities of highly abundant taxa and therefore increase the importance of the less abundant taxa in the analyses. The PERMANOVA test was conducted on Bray–Curtis similarity matrix and the residuals were permuted under a reduced model, with 9999 permutations. The null hypothesis was rejected when the

significance level p was <0.05 (if the number of permutation was lower than 150, the Monte Carlo permutation p was used). If significant differences were detected, these were examined using *a posteriori* pair-wise comparisons, using 9999 permutations under a reduced model. Afterwards, the similarity between mei fauna assemblages along the estuary, in the different sampling occasions, was plotted using non-metric multidimensional scaling (nMDS), with Bray–Curtis as similarity measure (Clarke and Green, 1988).

2.3.3. Nematodes assemblages

As the Nematoda was always the dominant mei fauna group, we decided to study this group in particular depth. Therefore, total density, genera diversity, trophic composition and several ecological indicators, either based on diversity (Margalef Index, d ; Shannon–Wiener diversity, H') or on ecological strategies (Index of Trophic Diversity, ITD; Maturity Index, MI), were calculated using the nematodes dataset, for each area and sampling occasion.

In order to investigate the trophic composition of the assemblages, marine nematodes genera were assigned to one of the four functional feeding groups, designated by Wieser (1953), based on buccal cavity morphology: selective (1A) and non-selective (1B) deposit feeders, epigrowth feeders (2A) and omnivores/predators (2B). The trophic classification of the freshwater nematodes was based on diet and buccal cavity structure information (Yeates et al., 1993; Traunspurger, 1997).

The Index of Trophic Diversity (Heip et al., 1985) was calculated as: $\text{ITD} = \sum \theta^2$, where θ is the density contribution of each trophic group to total nematode density, ranging from 0.25 (highest trophic diversity, i.e., each of the four trophic guilds account for 25% of the nematode density), to 1.0 (lowest trophic diversity, i.e., one trophic guild accounts for 100% of the nematode density). The Maturity Index (Bongers, 1990; Bongers et al., 1991) was used to analyze nematodes life strategy. Nematode genera were assigned a value on a scale (c – p score) accordingly their ability for colonizing or persisting in a certain habitat, from “colonizers” (c ; organisms with a high tolerance to disturbance events) to “persisters” (p ; low tolerance). Thus, the index is expressed as a c – p value, ranging from 1 (extreme colonizers) to 5 (extreme persisters) representing life-history characteristics associated with r - and K -selection, respectively (Bongers and Bongers, 1998; Bongers and Ferris, 1999) and varies from 1, under disturbed conditions, to 3 or 4, under undisturbed conditions. The index was calculated as the weighted average of the individual colonizer–persister (c – p) values as $\text{MI} = \sum_{i=1}^n \nu(i) \cdot f(i)$, where $\nu(i)$ is the c – p value of the taxon i and $f(i)$ is the frequency of that taxon.

Two-way permutational analyses of variance (PERMANOVA) were applied to test the null hypotheses that no significant spatial (between areas) and temporal (between sampling occasions) differences existed, in the nematode assemblage descriptors (total density, genera diversity, trophic composition, d , H' , ITD and MI). PERMANOVA was used as an alternative to ANOVA since its assumptions were not met, even after data transformation. Two-way PERMANOVA analyses were carried out with the same design described for mei fauna analysis. All PERMANOVA tests were conducted on Euclidean-distance similarity matrices and the residuals were permuted under a reduced model, with 9999 permutations. The null hypothesis was rejected when the significance level p was <0.05 (if the number of permutation was lower than 150, the Monte Carlo permutation p was used). Whenever significant differences were detected, these were examined using *a posteriori* pair-wise comparisons, using 9999 permutations under a reduced model.

In order to test for temporal and spatial differences regarding nematodes assemblages' composition, a two-way PERMANOVA analysis was carried out with the previously described design (“area”: 5 levels; “sampling occasion”: 6 levels), using Bray–Curtis as similarity measure. The null hypothesis was rejected when the significance level p was <0.05 (if the number of permutation

was lower than 150, the Monte Carlo permutation p was used). If significant differences were detected, these were examined using *a posteriori* pair-wise comparisons, using 9999 permutations under a reduced model. Nematode genera density data were first square root transformed in order to scale down densities of highly abundant genera and therefore increase the importance of the less abundant genera in the analyses, and the similarity between communities along the estuary, in the different sampling occasions, was plotted by non-metric multidimensional scaling (nMDS), using the Bray–Curtis similarity measure (Clarke and Green, 1988). Afterwards, the relative contribution of each genus to the average dissimilarities between areas and sampling occasions were calculated using two-way crossed similarity percentage analysis procedure (SIMPER, cut-off percentage: 90%).

2.3.4. Nematodes assemblages vs. environmental variables

The relationship between environmental variables and the structure of the nematodes community was explored by carrying out the BIOENV procedure (Clarke and Ainsworth, 1993), using Spearman's correlation.

3. Results

3.1. Environmental variables

Along the estuary, salinity and nutrient concentrations showed opposite trends, with higher salinity values and lower nutrient concentrations downstream and lower salinity values and higher nutrient concentrations upstream. A decrease in grain size was also observed from Oligohaline area toward the mouth of the estuary.

The PCA ordination of the environmental factors showed that the first two components (PC1, 29.0% and PC2, 23.8%) accounted for about 53% of the variability of the data (Fig. 2). The Oligohaline and Mesohaline samples were characterized by high nutrients concentration, at all sampling occasions, while in Autumn 2006, Winter 2007 and Spring 2007, the samples from these two upstream areas were clearly separated from the remaining ones mainly due to higher percentage of coarser sediments.

In general, independently from the sampling occasion, higher salinity, finer sediments and lower nutrient concentrations

characterized the samples from the Polyhaline NA, Polyhaline SA and Euhaline areas. With a few exceptions (mainly in Summer 2009), the two Polyhaline areas presented different environmental attributes: the Polyhaline NA samples having coarser sediments and the Polyhaline SA samples being characterized by finer sediments and higher OM content.

3.2. Meiofauna assemblages

Fourteen major taxa were identified along the estuary during the sampling period with Nematoda the dominant taxon (92.4%), followed by Polychaeta (4.7%) and Harpacticoid copepods (1.5%). All other taxa attained less than 1% [e.g. Bivalvia (0.4%), Oligochaeta (0.4%), Ostracoda (0.2%), Tardigrada (0.1%), Gastropoda (0.1%), Amphipoda (0.1%), Nauplii (0.1%)] and some taxa presented very low density (less than 0.03%), such as Ciliophora, Halacaroida, Turbellaria and Cladocera.

Total meiofauna density (\pm sd) ranged from 25.4 ± 25.9 ind 10 cm^{-2} (Oligohaline, Sp07) to 1383.5 ± 687.9 ind 10 cm^{-2} (Euhaline, Su06) and the number of taxa present varied from three (Mesohaline, Sp07; Euhaline, Au06 and Au09) to eleven (Polyhaline SA and Euhaline in Su06), with no clear increase from Oligohaline to Euhaline areas (Table 1).

PERMANOVA analysis of meiofauna assemblage composition data showed a significant interaction between “area” and “sampling occasion” (Table 2A). The Oligohaline area was different from all others on all sampling occasions, with minor exceptions in Au06 (Oligohaline similar to Euhaline, $t = 1.35$, $p = 0.143$), in Wi07 (Oligohaline only different from the Polyhaline SA, $t = 2.94$, $p = 0.002$) and in Sp07 (Oligohaline similar to Mesohaline, $t = 1.57$, $p = 0.104$). This pattern is distinctly visible in the nMDS ordination (Fig. 3), with a clear separation of Oligohaline and Mesohaline areas from the remaining ones.

3.3. Nematodes assemblages

3.3.1. Structure and trophic composition

The density (N) of nematodes ranged from 21.4 ± 23.5 ind 10 cm^{-2} in the Oligohaline area (Sp07) to 1323.1 ± 674.7 ind 10 cm^{-2} in the Euhaline area (Su06). Over the whole estuary, mean

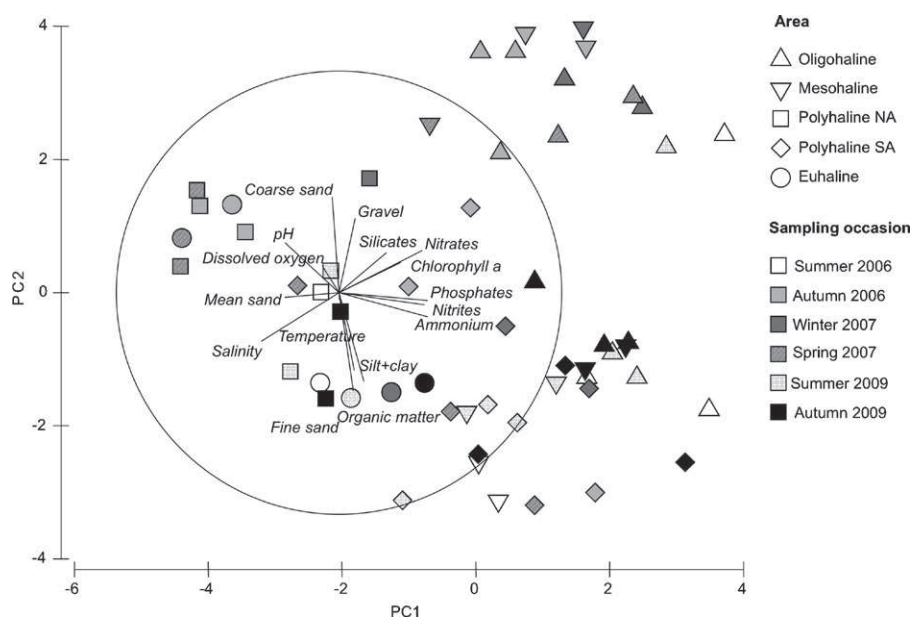


Fig. 2. Principal Component Analysis (PCA) plot based on the environmental variables measured in each “area” (Oligohaline, Mesohaline, Polyhaline North Arm, Polyhaline South Arm and Euhaline) and “sampling occasion” (Summer 06, Autumn 06, Winter 07, Spring 07, Summer 09 and Autumn 09). PC1 = 29.0%, PC2 = 23.8%.

Table 1
Mean density \pm standard deviation (number of individuals per 10 cm²) of meiofaunal taxa in each area (Oligohaline, Mesohaline, Polyhaline South Arm and Euhaline) and sampling occasion (Summer 2006, Su06; Autumn 2006, Au06; Winter 2007, W107; Spring 2007, Sp07; Summer 2009, Su09 and Autumn 2009, Au09).

Area	Sampling occasion	Nematoda	Polychaeta	Copepoda	Bivalvia	Oligochaeta	Ostracoda	Gastropoda	Nauplii	Tardigrada	Amphipoda	Ciliophora	Halacaroida	Turbellaria	Cladocera	Total
Euhaline	Su06	1323.1 \pm 674.7	4.8 \pm 2.2	30.9 \pm 14.0	6.4 \pm 0.7	4.0 \pm 1.5	4.0 \pm 3.1	3.2 \pm 3.1	5.2 \pm 4.1	0.8 \pm 0.3	0.6 \pm 0.6	0.4 \pm 0.7	1383.5 \pm 687.9			53.4 \pm 20.5
	Au06	52.6 \pm 19.9	0.6 \pm 1.0	0.2 \pm 0.3												374.5 \pm 160.2
	W107	332.7 \pm 134.2	5.0 \pm 1.5	33.5 \pm 34.4		0.2 \pm 0.3	1.2 \pm 1.2	1.6 \pm 0.3								152.7 \pm 10.5
	Sp07	139.3 \pm 9.9	8.8 \pm 4.6	3.6 \pm 0.6		1.0 \pm 1.7										163.6 \pm 65.7
	Su09	157.5 \pm 63.4	0.6 \pm 1.0	2.8 \pm 2.4	1.0 \pm 1.3		0.2 \pm 0.3	1.2 \pm 1.2	0.2 \pm 0.3							109.0 \pm 26.2
	Au09	103.6 \pm 22.9		1.2 \pm 0.6			4.2 \pm 4.2									681.9 \pm 500.5
	Su06	617.0 \pm 468.7	42.3 \pm 22.8	14.2 \pm 18.3	0.6 \pm 0.5	0.4 \pm 0.5	5.9 \pm 8.4	0.1 \pm 0.1	0.1 \pm 0.1		0.1 \pm 0.2	1.1 \pm 1.0	0.1 \pm 0.1			189.0 \pm 161.8
	Au06	172.0 \pm 150.7	15.7 \pm 14.9	1.1 \pm 1.8			0.1 \pm 0.2									544.0 \pm 521.8
	W107	526.1 \pm 506.0	8.7 \pm 7.7	7.8 \pm 7.7	0.4 \pm 0.5	0.9 \pm 1.1		0.1 \pm 0.2	0.1 \pm 0.1							210.6 \pm 145.0
Polyhaline SA	Sp07	196.9 \pm 134.9	9.9 \pm 9.4	2.9 \pm 3.4	0.1 \pm 0.1	0.7 \pm 0.5					0.1 \pm 0.1					212.9 \pm 77.5
	Su09	201.2 \pm 81.0	7.8 \pm 1.5	2.4 \pm 2.3	0.3 \pm 0.3	0.1 \pm 0.1	1.1 \pm 0.9		0.1 \pm 0.1							194.3 \pm 69.2
	Au09	182.6 \pm 70.7	9.5 \pm 4.5	1.5 \pm 1.3	0.1 \pm 0.2		0.2 \pm 0.2	0.1 \pm 0.1		0.1 \pm 0.1						267.0 \pm 1.3
Polyhaline NA	Su06	238.4 \pm 13.6	16.8 \pm 10.4	4.0 \pm 4.0	1.2 \pm 0.6	3.2 \pm 2.8	0.1 \pm 0.1	1.0 \pm 1.4	0.4 \pm 0.3		1.8 \pm 3.6	0.1 \pm 0.1				262.4 \pm 16.0
	Au06	259.9 \pm 14.8	2.3 \pm 1.3		0.1 \pm 0.1			0.1 \pm 0.1								75.5 \pm 106.7
	W107	72.8 \pm 103.0	1.7 \pm 2.4	0.3 \pm 0.3		0.4 \pm 0.6		0.1 \pm 0.1	0.1 \pm 0.1							184.0 \pm 108.1
	Sp07	173.5 \pm 98.3	10.1 \pm 9.5	0.2 \pm 0.3		0.1 \pm 0.1										313.2 \pm 121.9
	Su09	303.7 \pm 115.9	2.4 \pm 0.0	1.1 \pm 1.3	0.1 \pm 0.1	5.7 \pm 7.5		0.1 \pm 0.1								252.3 \pm 97.9
	Au09	247.0 \pm 100.2	2.4 \pm 2.8	0.7 \pm 1.0	0.2 \pm 0.3	1.9 \pm 1.8										252.9 \pm 28.7
	Su06	183.8 \pm 1.7	63.8 \pm 24.4	2.2 \pm 2.6	0.5 \pm 0.4	0.5 \pm 0.7		1.2 \pm 0.3		0.2 \pm 0.0	0.3 \pm 0.4	0.1 \pm 0.1	0.2 \pm 0.3			263.0 \pm 15.2
	Au06	260.5 \pm 16.5	2.0 \pm 0.6			0.1 \pm 0.1		0.1 \pm 0.1		0.2 \pm 0.3						216.2 \pm 105.7
	W107	209.8 \pm 106.3	5.4 \pm 0.3	0.2 \pm 0.3	0.2 \pm 0.3	0.1 \pm 0.1	0.5 \pm 0.7									70.6 \pm 75.5
Mesohaline	Sp07	68.0 \pm 74.6	2.4 \pm 1.1	0.2 \pm 0.3												64.0 \pm 44.6
	Su09	55.6 \pm 43.7	6.0 \pm 1.7	0.5 \pm 0.1	0.3 \pm 0.4	1.6 \pm 2.3				1.1 \pm 1.0						58.3 \pm 17.2
	Au09	55.1 \pm 17.5	1.0 \pm 0.0	0.6 \pm 0.9		0.5 \pm 0.4										129.7 \pm 43.5
Oligohaline	Su06	85.8 \pm 41.4	29.2 \pm 11.7	1.5 \pm 1.3	12.3 \pm 18.8	0.5 \pm 0.8	0.1 \pm 0.1		0.2 \pm 0.2							268 \pm 7.5
	Au06	23.9 \pm 6.0	0.7 \pm 0.6	0.1 \pm 0.1		1.9 \pm 3.2										78.2 \pm 98.5
	W107	67.4 \pm 82.0	4.5 \pm 6.9	0.3 \pm 0.4	0.3 \pm 0.6	0.1 \pm 0.1		0.1 \pm 0.1	5.4 \pm 9.4		0.1 \pm 0.1					25.4 \pm 25.9
	Sp07	21.4 \pm 23.5	1.9 \pm 2.3	1.6 \pm 0.9		0.2 \pm 0.3	0.2 \pm 0.2		0.1 \pm 0.1							36.6 \pm 19.5
	Su09	29.7 \pm 22.8	1.5 \pm 1.0	3.0 \pm 4.5	2.2 \pm 3.8	0.1 \pm 0.2										37.5 \pm 21.2
	Au09	32.6 \pm 17.3	1.2 \pm 0.2	0.7 \pm 0.5	0.1 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.2	0.1 \pm 0.1	0.1 \pm 0.1	2.5 \pm 3.6						

Table 2

Details of the two-factor PERMANOVA test (“area” with 5 levels, and “sampling occasion” with 6 levels, as fixed factors) for all variables analyzed. Bold values stand for the significant differences ($p < 0.05$). A – meiofauna composition; B – nematodes descriptors.

	Source of variation	Degrees of freedom	Sum of squares	Mean squares	Pseudo-F	P (perm)
A. Meiofauna Composition	Area	4	39,752	9937.9	16.28	0.0001
	Sampling occasion	5	23,716	4743.3	7.77	0.0001
	Area × sampling occasion	19	24,391	1283.7	2.10	0.0001
	Residual	139	84,871	610.58		
	Total	167	175,020			
B. Nematodes Total density	Area	4	2,423,900	605,970	24.31	0.0001
	Sampling occasion	5	2,012,300	404,860	16.24	0.0001
	Area × sampling occasion	19	4,162,200	219,060	8.79	0.0001
	Residual	139	3,464,500	24,925		
	Total	167	10,996,000			
Number of genera	Area	4	471.19	117.8	10.37	0.0001
	Sampling occasion	5	318.13	63.626	5.60	0.0001
	Area × sampling occasion	19	373.84	19.676	1.73	0.0401
	Residual	139	1578.6	11.357		
	Total	167	2823.6			
Trophic composition	Area	4	19,645	4911.3	8.10	0.0001
	Sampling occasion	5	19,402	3880.4	6.40	0.0001
	Area × sampling occasion	19	22,170	1166.9	1.92	0.0006
	Residual	139	84,261	606.2		
	Total	167	150,940			
Composition	Area	4	98,388	24,597	16.37	0.0001
	Sampling occasion	5	37,623	7524.6	5.01	0.0001
	Area × sampling occasion	19	61,000	3210.5	2.14	0.0001
	Residual	139	208,840	1502.4		
	Total	167	420,420			
Margalef Index	Area	4	48,505	12,126	21.99	0.0001
	Sampling occasion	5	4,5976	0.91952	1.67	0.152
	Area × sampling occasion	19	19,238	1.0125	1.84	0.025
	Residual	139	76,665	0.55154		
	Total	167	155.88			
Shannon-Wiener Index	Area	4	13.633	3.4082	8.22	0.0001
	Sampling occasion	5	2,0816	0.41632	1.00	0.4157
	Area × sampling occasion	19	11,831	0.62267	1.50	0.0972
	Residual	139	57,633	0.41462		
	Total	167	87,925			
Index of Trophic Diversity	Area	4	0.31339	0.078347	3.05	0.0203
	Sampling occasion	5	0.11341	0.022682	0.88	0.4951
	Area × sampling occasion	19	0.59974	0.031565	1.23	0.2383
	Residual	139	3.5658	0.025653		
	Total	167	4.5852			
Maturity Index	Area	4	4.1698	1.0425	9.86	0.0001
	Sampling occasion	5	0.99525	0.19905	1.88	0.1054
	Area × sampling occasion	19	3.5231	0.18543	1.75	0.0438
	Residual	139	14.701	0.10576		
	Total	167	24.568			

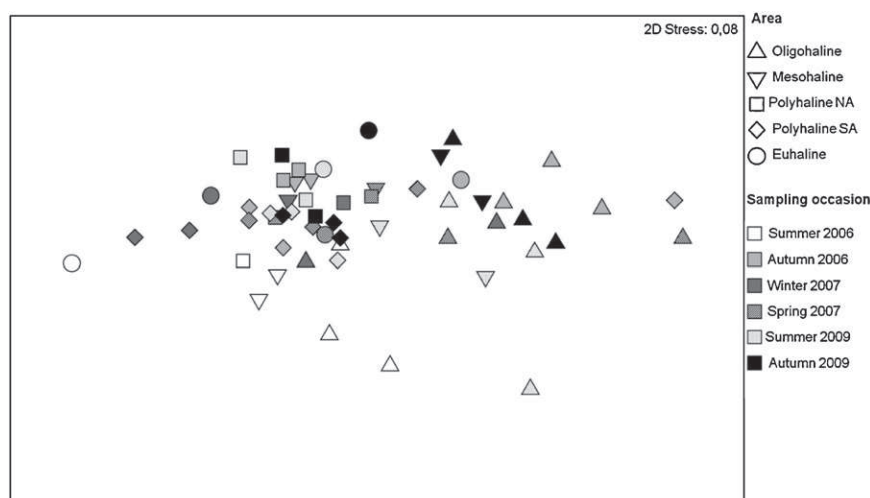


Fig. 3. nMDS ordination based on meiobenthos in each of the sampling stations in each “area” (Oligohaline, Mesohaline, Polyhaline North Arm, Polyhaline South Arm and Euhaline) and “sampling occasion” (Summer 06, Autumn 06, Winter 07, Spring 07, Summer 09 and Autumn 09).

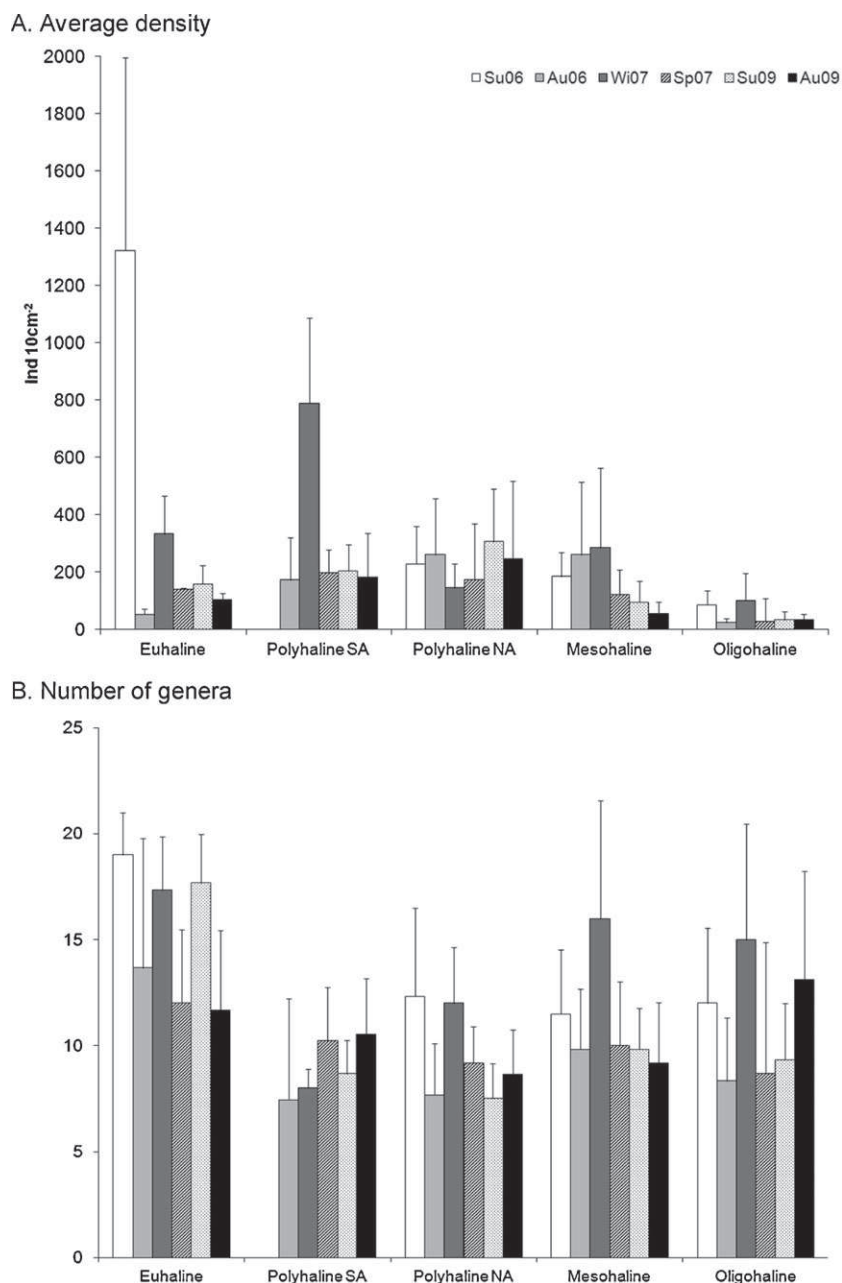


Fig. 4. Nematode community in each “area” (Oligohaline, Mesohaline, Polyhaline North Arm, Polyhaline South Arm and Euhaline) during the study period (Su06, Summer 2006; Au06, Autumn 2006; Wi07, Winter 2007; Sp07, Spring 2007; Su09, Summer 2009; Au09, Autumn 2009). (A) Average density (ind 10 cm⁻²); (B) number of genera (S).

density (\pm sd) was highest in Wi07 (363.40 ± 343.16 ind 10 cm⁻²), and lowest during Au09 (123.04 ± 154.79 ind 10 cm⁻²). Generally, the highest densities were reached in the Euhaline and Polyhaline areas (Fig. 4A). PERMANOVA analysis of density data showed a significant interaction between “area” and “sampling occasion” (Table 2B). Individual pair-wise comparisons on interaction factor (“area” \times “sampling occasion”) showed that the Oligohaline area, in general, showed significantly lower density values than the other areas, regardless of the sampling occasion. Moreover, the Polyhaline NA did not show significant differences through time while all other areas showed significant differences in density between one or more sampling occasions (see Supplementary material – Annex).

Nematodes accounted for between 88% (Su06) and 95% (Au06) of the total meiofaunal density and a total of 106 nematode genera, belonging to 40 families, were identified along the estuary during

the study period. The most abundant orders were Chromadorida (46.3%), Monhysterida (36.7%) and Enoplida (11.7%) and the most abundant families were Comesomatidae (25.3%), Xyalidae (16.7%), Linhomoeidae (11.8%), Chromadoridae (10.3%) and Sphaerolaimidae (8.6%).

The number of genera (S) ranged between 8 in the Polyhaline NA area (Su09) and 19 in the Euhaline area (Su06) (Fig. 4B). PERMANOVA revealed a significant interaction of factors “area” and “sampling occasion” for the number of genera (Table 2B). The pair-wise tests performed on the interaction term showed that in Au06, Sp07 and Au09 there were no significant differences in number of genera between areas, while in the remaining sampling occasions the Euhaline area showed higher diversity than the other areas. All areas showed significant variation in the number of genera between at least two sampling occasions (see Supplementary material – Annex).

Table 3

Average density (\bar{x} ; number individuals per 10 cm²), percentage of contribution (%) and rank by density (Rk) of nematode genera in each area of the Mondego estuary derived from pooled data from all sampling occasions. Table only lists the genera that contributed >0.5% to the total density and the five most abundant genera in each area are bolded.

Genera	Total average density	%	Euhaline			Polyhaline SA			Polyhaline NA			Mesohaline			Oligohaline		
			\bar{x}	%	Rk	\bar{x}	%	Rk	\bar{x}	%	Rk	\bar{x}	%	Rk	\bar{x}	%	Rk
<i>Sabatieria</i>	249.9	23.5	38.5	10.9	2	87.5	31.9	1	121.7	51.9	1	1.6	1.0	12	0.5	1.1	16
<i>Daptonema</i>	163.1	15.3	36.4	10.3	3	45.9	16.7	3	22.2	9.5	3	47.6	29.8	1	11.1	25.8	1
<i>Terschellingia</i>	86.7	8.2	7.3	2.1	13	49.3	18.0	2	7.8	3.3	7	21.6	13.5	3	0.8	1.8	12
<i>Metachromadora</i>	86.0	8.1	76.7	21.8	1	5.6	2.0	9	3.6	1.5	10	0.1	0.0	45	0.1	0.1	47
<i>Sphaerolaimus</i>	84.6	8.0	18.9	5.4	6	34.9	12.7	4	22.2	9.5	2	8.5	5.3	6	0.1	0.3	30
<i>Anoplostoma</i>	75.5	7.1	25.3	7.2	5	9.5	3.5	6	10.2	4.3	5	28.2	17.7	2	2.2	5.2	4
<i>Dichromadora</i>	47.1	4.4	5.0	1.4	18	5.5	2.0	10	20.9	8.9	4	13.6	8.5	4	2.2	5.1	5
<i>Viscosia</i>	37.0	3.5	14.4	4.1	7	8.9	3.3	7	9.1	3.9	6	4.3	2.7	8	0.3	0.7	19
<i>Ptycholaimellus</i>	35.4	3.3	8.4	2.4	11	10.5	3.8	5	4.8	2.1	8	10.4	6.5	5	1.3	3.1	7
<i>Microlaimus</i>	30.1	2.8	29.7	8.4	4				0.4	0.2	16				0.0	0.1	56
<i>Linhomoeus</i>	18.5	1.7	8.8	2.5	10	7.7	2.8	8	1.8	0.8	11	0.1	0.0	38	0.1	0.3	28
<i>Axonolaimus</i>	14.4	1.4	11.1	3.2	8	0.1	0.0	26	0.5	0.2	14	1.6	1.0	11	1.0	2.4	9
<i>Paracyatholaimus</i>	13.4	1.3	2.4	0.7	22	0.1	0.0	28	0.3	0.1	17	7.4	4.6	7	3.3	7.6	3
<i>Mesodorylaimus</i>	12.5	1.2	0.4	0.1	35							2.8	1.7	10	9.4	21.8	2
<i>Prochromadorella</i>	11.0	1.0	10.7	3.0	9	0.1	0.0	27	0.1	0.1	23						
<i>Leptolaimus</i>	8.4	0.8	1.5	0.4	27	0.6	0.2	16	4.5	1.9	9	1.4	0.9	13	0.4	0.9	18
<i>Molgolaimus</i>	8.0	0.8	7.7	2.2	12	0.2	0.1	25	0.1	0.0	28	0.1	0.0	40			
<i>Calyptronema</i>	7.0	0.7	6.4	1.8	14	0.7	0.2	15									
<i>Chromadora</i>	6.4	0.6	5.2	1.5	16	0.6	0.2	17	0.5	0.2	13						
<i>Spilophorella</i>	5.6	0.5				0.2	0.1	23	1.7	0.7	12	3.5	2.2	9	0.1	0.3	29
<i>Aegialolaimus</i>	5.5	0.5	5.5	1.6	15												
<i>Halalaimus</i>	5.4	0.5	3.9	1.1	20	0.9	0.3	13	0.3	0.1	18	0.2	0.1	26	0.2	0.4	26
<i>Paralinhomoeus</i>	5.2	0.5	5.1	1.4	17							0.1	0.0	41			
<i>Oncholaimellus</i>	5.0	0.5	4.7	1.3	19							0.2	0.1	29	0.1	0.1	40
Other genera	41.3	3.9	17.8	5.1		5.3	1.9		1.9	0.8		6.5	4.1		9.8	22.8	
Mean density			351.7			274.0			234.8			159.6			43.0		
Total genera number			53			33			35			58			84		

Throughout the study period, fifteen genera dominated the nematode assemblages (90.8%): *Sabatieria*, *Daptonema*, *Terschellingia*, *Metachromadora*, *Sphaerolaimus*, *Anoplostoma*, *Dichromadora*, *Viscosia*, *Ptycholaimellus*, *Microlaimus*, *Linhomoeus*, *Axonolaimus*, *Paracyatholaimus*, *Mesodorylaimus* and *Prochromadorella* (Table 3). The remaining genera all represented abundances lower than 1%. The most spatially widespread genus was *Daptonema* (present along the whole length of the estuary through the entire sampling period), followed by *Sabatieria* and *Dichromadora* (Table 3). Freshwater nematodes comprised 3.5% of the total nematodes density (1% in Sp07 to 4.4% in Wi07).

The five dominant genera showed clear variation over the study period, as shown in Fig. 5, and a distinct pattern of genera turnover along the estuary is visible. Non-selective deposit feeders (1B) like *Sabatieria* and *Daptonema*, showed an opposite density contribution trend in the Polyhaline areas, with the contribution of *Sabatieria* increasing from Wi07 to Au09, and *Daptonema* decreasing in the same period. *Sabatieria* was almost absent in the Mesohaline and Oligohaline areas, where *Daptonema* showed a high contribution. *Terschellingia*, a selective deposit feeder (1A), showed high contributions in Wi07, especially in the Polyhaline SA and Mesohaline areas. Predators (2B), like *Metachromadora* and *Sphaerolaimus*, peaked on different sampling occasions, with a high contribution of *Metachromadora* in the Euhaline area, while *Sphaerolaimus* was mostly observed in the Polyhaline NA (Au06) and Mesohaline (Wi07) areas.

Throughout the estuary, the nematodes community was characterized by a dominance of non-selective deposit feeders (52.0 ± 12.1%) during the entire study period, followed by omnivores/predators (23.2 ± 8.1%), epigrowth feeders (15.9 ± 3.3%) and selective deposit feeders (8.9 ± 4.8%). Non-selective deposit feeders were the most abundant trophic group, in almost all areas and sampling occasions, ranging from 22.5% (Euhaline area, Au06) to 81.6% (Polyhaline NA area, Au09). In the Mesohaline and

Oligohaline areas there was a lower contribution of predators on all sampling occasions (ranging from 1.7% in Au06 to 16.6% in Wi07, both in the Mesohaline area) compared with the remaining areas (ranging from 7.3% in Au09, Polyhaline NA area to 56.7% in Au06, Euhaline area) (Fig. 6). PERMANOVA analysis of trophic structure data showed a significant interaction between factor “area” and “sampling occasion” (Table 2B). Individual pair-wise comparisons performed on the interaction factor showed significant differences in trophic composition between areas on all sampling occasions and also significant differences at each area throughout the study period (see Supplementary material – Annex).

Regarding the overall composition, multivariate PERMANOVA analysis showed that the estuarine assemblages were different between areas and sampling occasions (Table 2B). In concrete, depending on the chosen area, there were significant differences between distinct pair of sampling occasions. The results are supported by a visual assessment of the patterns in the nMDS ordination of square-root transformed data, using Bray–Curtis, as shown in Fig. 7.

Two-way SIMPER analysis showed how the nematodes genera contributed to similarity values of the a priori defined groups. Maximum dissimilarities were obtained between the Oligohaline area and both the Polyhaline areas (80.15% with Polyhaline SA and 79.57% with Polyhaline NA) and Euhaline area (79.78%). Maximum dissimilarities were also observed between Summer 06 and the following three sampling occasions, Autumn 06 (71.57%), Winter 07 (68.59%) and Spring 07 (68.58%). The genera that contributed most to the similarity within both sampling occasions and areas were *Daptonema*, *Sabatieria*, *Sphaerolaimus* and *Dichromadora*.

3.3.2. Indices estimation

Margalef Index (d) and Shannon-Wiener index values (H') (Fig. 8A), followed the trend shown by the number of genera (Spearman correlation = 0.74 and 0.72, respectively; $p < 0.05$). The

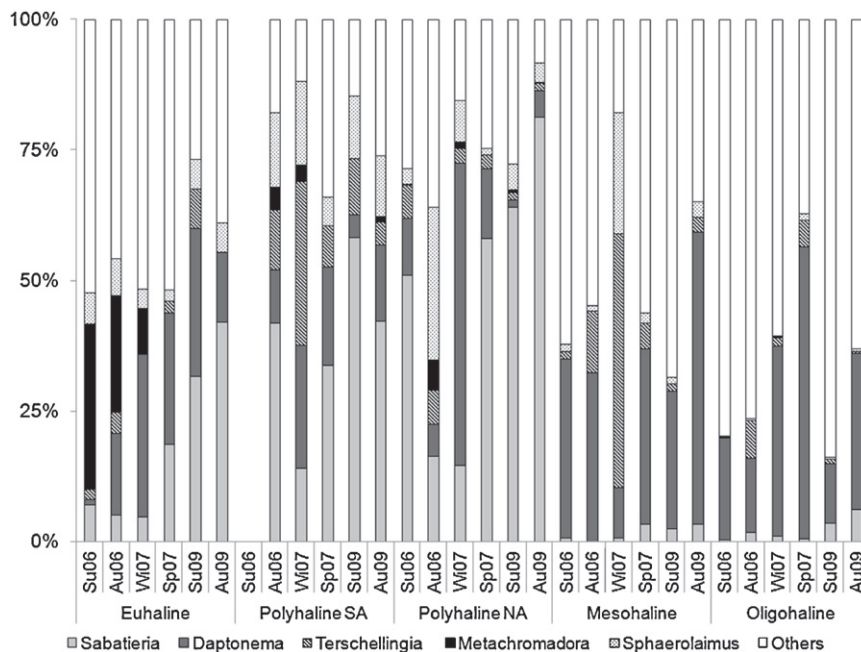


Fig. 5. Percentage of contribution of the five most abundant nematode genera (*Sabatieria*, *Daptonema*, *Terschellingia*, *Metachromadora* and *Sphaerolaimus*) in each “area” (Oligohaline, Mesohaline, Polyhaline North Arm, Polyhaline South Arm and Euhaline) and “sampling occasion” (Summer 06, Autumn 06, Winter 07, Spring 07, Summer 09 and Autumn 09).

Margalef Index showed a significant interaction between “area” and “sampling occasion” (Table 2B). The Mesohaline and Euhaline areas did not show significant differences in richness throughout the study period, while the Oligohaline area showed several pairs of sampling occasions with significantly different richness values, higher in Wi07 and Au09. Moreover, no significant differences were found between areas in Su06 and Sp07 (see Supplementary material – Annex). The Shannon-Wiener index

showed significant differences between all pairs of areas (Table 2B) except between Oligohaline–Mesohaline ($t=1.27$, $p=0.21$) and Mesohaline–Polyhaline SA ($t=1.24$; $p=0.22$). In general, both indicators showed a lower diversity in the Polyhaline areas (Fig. 8A).

The Index of Trophic Diversity ranged from 0.31 (Euhaline, Su06) to 0.62 (Polyhaline NA, Sp07). Significant differences were observed between areas (Table 2B), with higher values in the Oligohaline and Mesohaline areas, indicating lower trophic diversity,

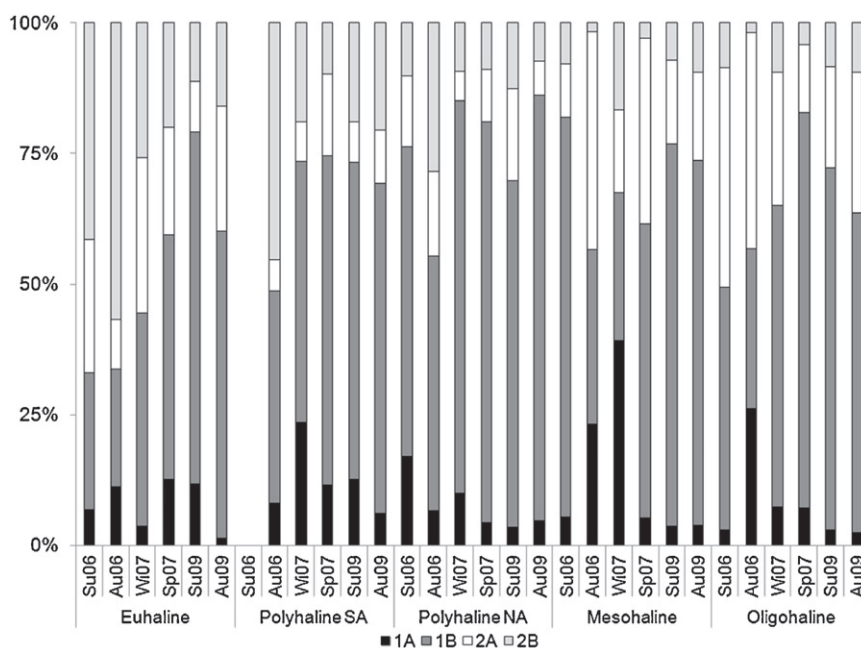


Fig. 6. Percentage of contribution of the different trophic groups, in each “area” (Oligohaline, Mesohaline, Polyhaline North Arm, Polyhaline South Arm and Euhaline) and “sampling occasion” (Summer 06, Autumn 06, Winter 07, Spring 07, Summer 09 and Autumn 09). 1A – selective deposit feeders; 1B – non-selective deposit feeders; 2A – epigrowth feeders; 2B – omnivores/predators.

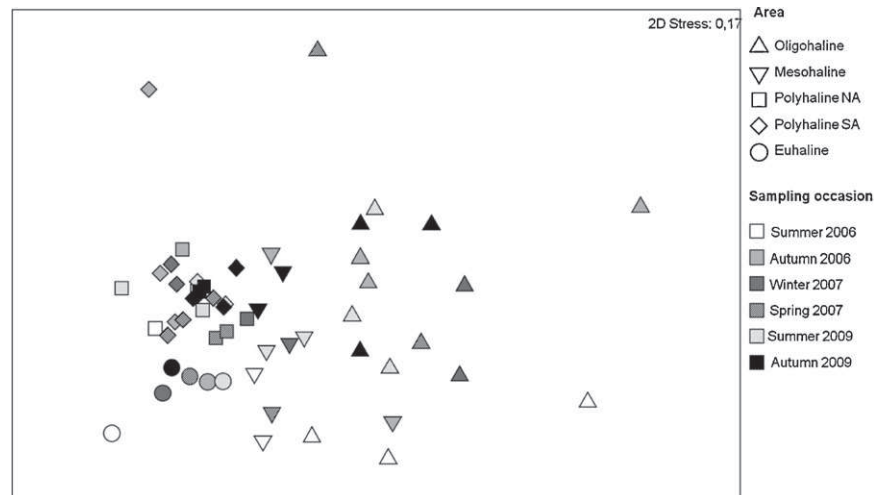


Fig. 7. nMDS ordination based on nematodes dataset in each “area” (Oligohaline, Mesohaline, Polyhaline North Arm, Polyhaline South Arm and Euhaline) and “sampling occasion” (Summer 06, Autumn 06, Winter 07, Spring 07, Summer 09 and Autumn 09).

Table 4

BIOENV results carried out for nematodes assemblages and environmental data, in each sampling occasion.

Sampling occasion	Spearman's rank correlation	Variables
Summer 2006	0.938	Salinity, NO_3^- , mean sand, coarse sand, Chl <i>a</i>
Autumn 2006	0.245	pH, fine sand, coarse sand
Winter 2007	0.636	Salinity, pH, mean sand
Spring 2007	0.839	Salinity, NO_3^-
Summer 2009	0.862	Salinity, NO_3^-
Autumn 2009	0.642	NO_3^- , silicates, %OM, mean sand

and lower values in the Polyhaline and Euhaline areas (Polyhaline NA > Polyhaline SA, Polyhaline NA > Euhaline), indicative of a higher trophic diversity (Fig. 8B).

The Maturity Index (MI) ranged between 2.1 (Polyhaline NA in Wi07, Sp07, Su09 and Au09; Mesohaline in Su06 and Sp07) and 3.0 (Oligohaline, Su06) (Fig. 8B) and most nematodes showed a *c-p* value of 2 (average = 70%), followed by *c-p* values of 3 (26%). The MI showed a significant interaction between the factors “area” and “sampling occasion” (Table 2B). Individual pair-wise comparisons performed on the interaction revealed no seasonal differences in the Polyhaline SA area. The MI values of the Mesohaline area exhibited the highest temporal variations. Interestingly, in Au06 (flood period), no significant differences in MI were recorded along the estuary.

3.4. Environmental variables vs. nematode assemblages

Separate BIOENV analysis were performed for each sampling occasion in order to analyze the main factors responsible for the distribution of nematodes along the estuary in each sampling occasion, with salinity, grain size variables and nutrients always being correlated with the nematode assemblage composition (Table 4).

4. Discussion

The combination of the temporal and spatial information on meiofauna and nematodes of the Mondego estuary allowed a full description of the meiobenthic communities along the estuarine gradient to be made. The information was then analyzed in the

context of the ecological assessment of transitional waters using these communities, making available information on the ecological conditions of the system and initiating a baseline for long-term monitoring studies. Previous studies have only been focused on one season, lacking temporal replication (Alves et al., 2009; Adão et al., 2009; Patrício et al., 2012), and the present study, as well as integrating the complete estuarine gradient, was repeated on six sampling occasions, allowing a more extensive database to be analyzed and related to the environmental gradient.

The environmental characterization of the Mondego estuary was based on abiotic measurements collected at each sampling event. The characterization of a system based on chemical parameters only provides information about quality at the time of measurement, lacking the sensitivity to determine the impact of previous events on the ecology of the system (Spellman and Drinan, 2001). However, bioindicators provide indications about past conditions and accurately assess ecological conditions it is necessary to use a set of indicators which represent the structure, function and composition of the system. In this study, meiobenthic communities were studied in detail, with special emphasis on nematode assemblages.

A clear estuarine gradient, from the oligohaline area toward the euhaline zone was observed during the survey period, mainly caused by variations in salinity, nutrient concentrations and sediment grain size. The identification of both arms of the Mondego estuary as two different subsystems was confirmed, representing distinct hydrological regimes. Salinity increased from upstream toward the mouth of the estuary on all sampling occasions except in Autumn 2006. During this season, a period of heavy rain and flooding occurred (INAG source), lowering salinity values and confirming the importance of extreme events in changing the environmental characteristics of estuaries. The nematode community was affected at this time since the separation of salinity zones along the estuary was not so distinct. The severe flood may have caused sediment displacement and erosion as well as changing the interstitial water salinity (Santos et al., 1996), and organisms may have been washed away, leading to the low density values observed during this season.

Both salinity and sediment structure are major factors influencing meiobenthic community structure (Heip et al., 1985) and results from the BIOENV analysis showed that the distribution pattern of nematodes was mainly structured by distinct environmental factors like salinity, sediment grain size and water nutrients, supporting the primary influence of the estuarine gradient on

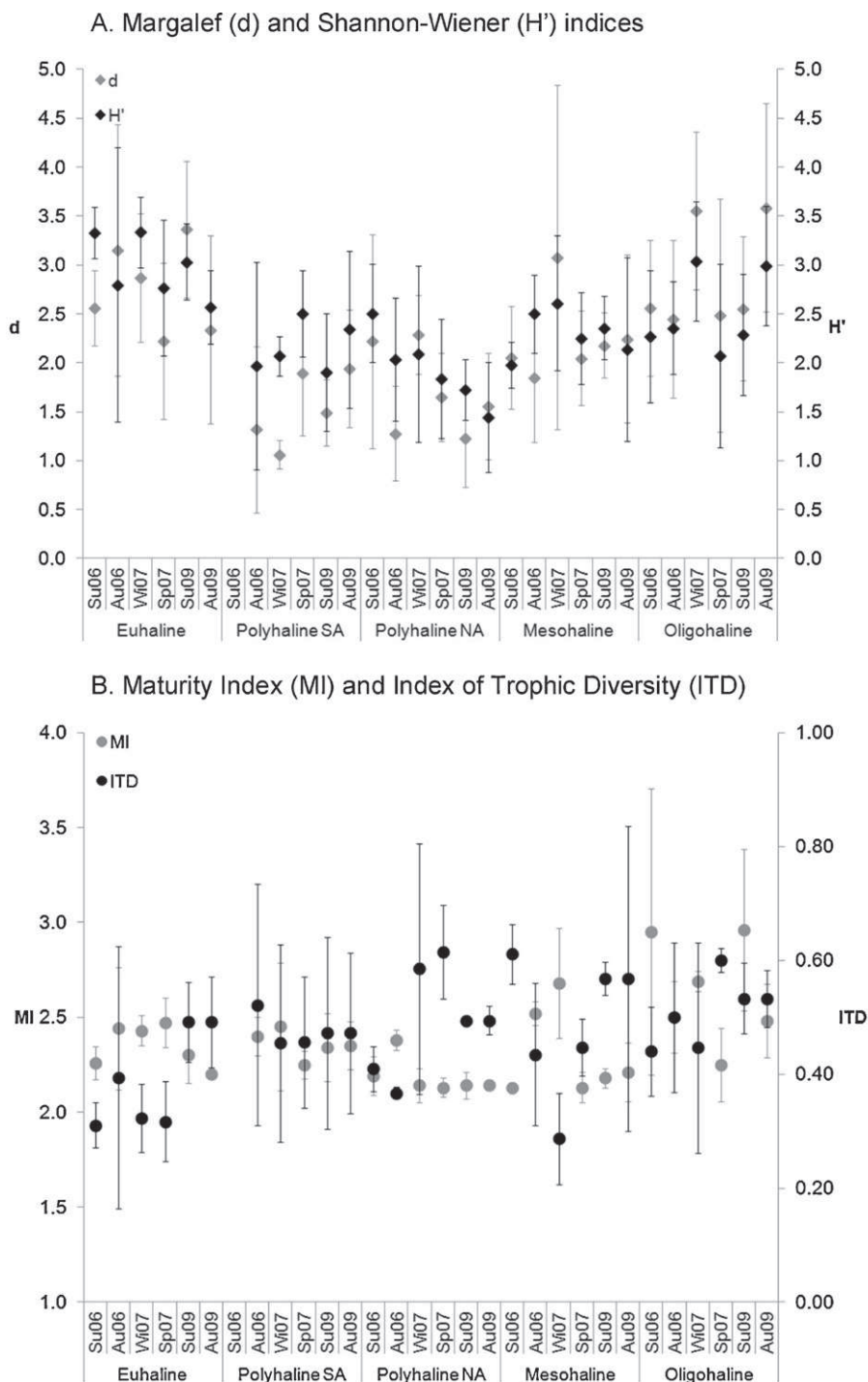


Fig. 8. Ecological indicators values in each “area” (Oligohaline, Mesohaline, Polyhaline North Arm, Polyhaline South Arm and Euhaline) and “sampling occasion” (Summer 06, Autumn 06, Winter 07, Spring 07, Summer 09 and Autumn 09). (A) Margalef Index ($d \pm$ standard deviation) and Shannon-Wiener index ($H' \pm$ standard deviation) (bits ind^{-1}); (B) Index of Trophic Diversity ($ITD \pm$ standard deviation) and Maturity Index ($MI \pm$ standard deviation).

nematode community patterns (Austen and Warwick, 1989; Vincx et al., 1990; Coull, 1999; Ferrero et al., 2008; Schratzberger et al., 2008; Adão et al., 2009). However, despite the other environmental differences between the polyhaline areas, the meiofauna and nematode communities were similar, emphasizing the prime importance of salinity in defining and limiting species distribution in transitional water systems (Austen and Warwick, 1989; Vincx et al., 1990; Soetaert et al., 1995; Attrill, 2002; Ferrero et al., 2008), its effects overriding that of sediment grain size composition (Austen and Warwick, 1989; Adão et al., 2009).

Meiofauna density and diversity were similar to other meiofauna communities, with densities falling within the range observed in other European estuaries (Smol et al., 1994; Soetaert et al., 1994, 1995). The dominance of nematodes over all other taxa is well documented, with Nematoda typically being the most abundant taxon (usually 60–90%) (Coull, 1999). Polychaeta ranked second, contrary to the common observation that copepods are usually more abundant (Coull, 1999). Harpacticoid copepods are sensitive to environmental perturbation (Hicks and Coull, 1983; Van Damme et al., 1984) and the low densities observed may

indicate anthropogenic disturbances in the Mondego estuary. Low density of harpacticoid copepods was also observed in the Westerschelde (Van Damme et al., 1984; Soetaert et al., 1995) and was ascribed to pollution effects.

The increase in taxonomic resolution (from meiofauna major taxa to nematode genus level) enhanced our knowledge of the system, suggesting that higher taxonomic resolution may be more informative for measurement of changes in meiofauna community structure. However, some studies of meiofauna communities as indicators of status in marine environments (Schratzberger et al., 2000) and as indicators of pollution in harbors (Moreno et al., 2008), for instance, have shown that meiofauna taxon assemblages could provide a sensitive and clear measure of environmental status when comparing inshore and offshore locations and that indicators based on meiofauna taxa demonstrated a significant correlation with the concentration of contaminants.

Nematodes communities comprised a high number of genera but with few dominant ones, as observed in other estuaries (Austen et al., 1989; Li and Vincx, 1993; Soetaert et al., 1995; Rzeznik-Orignac et al., 2003; Steyaert et al., 2003; Ferrero et al., 2008). The dominant genera were similar to those found in the Brouage mudflat (France) (Rzeznik-Orignac et al., 2003) and in the Thames estuary (United Kingdom) (Ferrero et al., 2008), indicating that species that are able to tolerate the highly variable salinity in estuaries tend to be abundant, taking advantage of the plentiful food resources of estuaries (Hourston et al., 2011). Also, the wide distribution range of *Daptonema*, *Sabatieria* and *Dichromadora*, also observed by Ferrero et al. (2008), reflects the wide salinity range tolerated by these genera (Heip et al., 1985; Moens and Vincx, 2000; Ferrero et al., 2008). Moreover, *Sabatieria*, *Daptonema* and *Terschellingia*, the three most abundant genera in the present study, are known to be tolerant to pollution (Soetaert et al., 1995; Austen and Somerfield, 1997; Schratzberger et al., 2006; Steyaert et al., 2007; Gambi et al., 2009; Armenteros et al., 2009), and their high densities along the Mondego estuary may be indicative of the pressures from which this estuary suffers. In fact, Moreno et al. (2011), in an evaluation of the use of nematodes as biological indicators of environmental quality in sediments of the Mediterranean Sea stated that the presence of some genera provided accurate information on the ecology and adaptation of organisms to environmental conditions. In this study, disturbed places were characterized by a high density of *Terschellingia*, *Paracomesoma* and *Sabatieria*, and sites classified as in moderate or poor ecological quality status were also dominated by *Daptonema*, indicating that such inhospitable habitat conditions can only be tolerated by genera able to thrive in extreme conditions (Moreno et al., 2008).

Genera diversity broadly followed the Remane's diagram (1934) for the effect of the salinity gradient on benthic invertebrates species richness (postulated for the Baltic Sea), with high diversity in the more stable marine and freshwater waters. According to Attrill (2002), salinity variation over time may be more important than average salinity for the distribution of nematodes along the estuary (also confirmed by Ferrero et al., 2008).

The premise that environmental variables influence meiobenthic communities is well described, but the question of how far back we should consider the environmental history of a system in order to explain the distribution of the communities depends on the life-history characteristics of the species and, coupled with the characterization of the environment, extreme events should also be taken in consideration (Soetaert et al., 1995).

Spatial variability, with the transition between areas being characterized by different assemblages and with strong variations in genera dominance, was detected. The shift from an oligohaline nematode community, characterized by low density, high nematode diversity and high abundance of *Daptonema*, to a typical estuarine community, characterized by high nematode density,

was observed, as in the Thames estuary (Ferrero et al., 2008). The remaining areas were also discrete, each one characterized by a different community, with the exception of the Polyhaline areas (see above).

In the present study, besides the clear spatial pattern, some temporal variations were also observed. Similar results were observed in the Swan River estuary, Australia (Hourston et al., 2009), with nematode species being markedly influenced by both site and season, with site being the most important factor. In temperate regions, nematode densities usually peak in the warmest months (Hicks and Coull, 1983; Smol et al., 1994) and in this study, although the highest density was observed in Summer 2006, the pattern was not repeated in the other warm seasons.

The multivariate analysis allowed a representation of both environmental and biological (meiofauna and nematodes) data, showing that the estuarine abiotic gradient was mostly reflected in the biological communities.

Spatial and temporal variations of nematode assemblages has been studied in several systems (e.g. Yodnarasri et al., 2008; Armenteros et al., 2009; Hourston et al., 2009, 2011; Semprucci et al., 2010) and, in order to use that information for ecological assessment, the application of ecological indices to the nematodes assemblages enhanced our knowledge on the benthic environment. Coupled with the taxonomic diversity, functional diversity is important for interpreting distribution patterns of the communities (Schratzberger et al., 2008). In what refers to meiobenthic communities, and besides the common diversity measures, specific indicators rely on nematodes information, such as the Maturity Index and the Index of Trophic Diversity. These two indices do not depend on the system, not suffering from lack of generality and the use of indicators based on different ecological principles is, according to Dauer et al. (1993) highly recommended in determining the environmental quality status of an ecosystem (Marques et al., 2009).

Knowing that the Mondego estuary suffers from anthropogenic pressures, especially in the Polyhaline areas (Northern arm – dredging activities, harbor; Southern arm – inputs from the Pranto River and agricultural runoffs), we can evaluate the performance of the indices in differentiating homogeneous sectors of impact along the estuary. The results verified that the indices behaved differently. For example, the Index of Trophic Diversity, generally used to correlate trophic diversity with pollution levels (Heip et al., 1985), appeared only to differentiate “extreme” conditions such as the relatively good ecological conditions in the mouth of the estuary (reflected in high trophic diversity index values) and the upstream part of the estuary having lower ecological status. In the upstream zone, the incorporation of feeding information on the freshwater genera, mostly predators, may have contributed to the observed pattern. However, if this dominance is a natural feature in estuaries, the parameters of this index should be readjusted so that the predominance of freshwater nematodes does not exclusively imply a classification of bad ecological conditions. A similar result was observed by Moreno et al. (2011), with the ITD not separating sites with different ecological classifications and even indicating a good Ecological Quality Status in disturbed sites.

Furthermore, the classification of feeding complexity, as first described by Wieser (1953), has the disadvantage of confining nematode species to a single trophic status (Heip et al., 1985), which may not represent the real complexity of feeding habitats of nematodes (Moens and Vincx, 1997), with trophic plasticity being described for most feeding types (Moens et al., 2005; Schratzberger et al., 2008).

On the other hand, the low Maturity Index values observed in both the polyhaline and euhaline areas suggested a high stress level, since opportunistic genera increase in abundance in adverse conditions (Bongers and Bongers, 1998; Gyedu-Ababio and Baird, 2006).

An opposite trend was observed in the oligohaline area, where the MI reached maximum values, indicating a better ecological status, with the MI also capturing the composition variations that occurred in the upstream area over time (higher dispersion of oligohaline samples in the nMDS). These observations may be related to the origin of the index which, contrary to the Index of Trophic Diversity, was developed for soil and freshwater nematodes (Bongers and Bongers, 1998) and lately extended to assessing the condition of marine and brackish sediments, being less frequently applied to marine nematodes (Bongers et al., 1991), partly due to a lack of empirical support for the classification of some marine genera and the absence or rarity of extreme colonizers and persisters in most marine habitats (Schratzberger et al., 2006). According to Moreno et al. (2011), the analysis of the percentage composition of the different *c-p* classes in each site allowed a better classification of the studied sites than the application of the MI.

This study emphasized the need for the development of a nematode-based multimetric index (Patrício et al., 2012), taking in consideration density, composition, and genera sensitivity/tolerance to stress, as proposed by Moreno et al. (2011). Moreover, this multimetric index should include information with parameters more accurately based on marine/estuarine nematodes including maturity and trophic values specifically calculated for the genera. There is also the need for re-evaluation of the boundaries of the indices used, as an index can provide a good characterization of the system but may be limited to a specific spatial area. The correct application of nematode information and its integration into a multimetric index, with a suitable combination of several indicators, would provide clearer information regarding ecosystem status, since it would overcome the limitations of individual analyses. It is also important to bear in mind that the evaluation of reference conditions in order to provide comparisons with disturbed environments is usually required. Since meiobenthic studies are quite recent in Portuguese estuaries, it may be interesting to determine if the analysis of meiobenthic communities in an estuary where human perturbations are almost absent (Mira estuary – Alves et al., 2009; Adão et al., 2009) may be used in the establishment of reference conditions.

Acknowledgements

The present study was carried out using funds provided by the research projects RECONNECT (PTDC/MAR/64627/2006) and 3M-RECITAL (LTER/BIA-BEC/0019/2009). Additionally, it benefited from one grant, given by FCT – (SFRH/BD/62000/2009). The study was also subsidized by the European Social Fund and MCTES national funds, through the QREN and POPH: Human Potential Operational Programme – NSRF: National Strategic Reference Framework – 4.4. A special thanks to all who assisted us during field and laboratory work. The senior author (J. Patrício) provided a major contribution as project's leader.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2012.07.013>.

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