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Spatial and temporal distribution of harpacticoid copepods in Mondego estuary

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*Seasonal and spatial variations in the composition and relative contribution of the harpacticoid species in the Mondego estuary (western Portugal) were studied based on a monthly sampling along a salinity gradient. These benthic harpacticoids were collected in the water column by means of a 63-μm plankton net. The influence of hydrological parameters (temperature, salinity, oxygen dissolved concentration, pH, turbidity, chlorophyll-*a*, total suspended solids and nutrient concentrations) was analysed by means of a redundancy data analysis. In addition, this is the first study to provide a checklist of benthic harpacticoids from this estuary. In total, 13 species plus six species not yet identified but known to belong to the genus* Canuella, Microsetella, Ectinosoma, Mesochra, Harpacticus *and* Parapseudoleptomesochra *were identified. Copepodites and adults of* Euterpina acutifrons *and* Paronychocamptus nanus *were most abundant in this harpacticoid community. While* P. nanus *dominated in winter and spring at upstream stations,* E. acutifrons *dominated more downstream. Although this species occurred along the whole salinity gradient, its highest abundances were found in the north arm of the estuary, except in autumn. This distribution may be due to the adaptability of* E. acutifrons *to different environments and the intermediate position between marine and estuarine conditions assigned to this species.* Paraleptastacus *cfr.* spinicauda *showed a relative occurrence of 5–10% in all stations, except at the mouth of the estuary (M).* Tachidius discipes *was found in low densities in Mondego estuary in spite of the large numbers of copepodites of this species in the southern arm, characterized by a high level of total suspended solids. The northern arm of the estuary was characterized by a higher numerical occurrence of harpacticoid organisms throughout the study years, with the higher densities towards the freshwater part (upstream areas).* Canuella *sp.,* Ectinosoma *sp. (copepodite),* Ectinosoma melaniceps, Leptocaris brevicornis, Phyllognathopus viguieri, Microsetella norvegica *(copepodite) and* Macrosetella gracilis *(copepodite) were considered rare species. Nonetheless harpacticoid species were represented by a higher number of adults in the northern arm, and juveniles in the southern arm.*

Keywords: Harpacticoida seasonal and spatial variation, hydrological parameters, estuary

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INTRODUCTION

Copepods are the most common and widely distributed group of small aquatic crustaceans. Copepods have an important role in the food web, linking primary producers and higher trophic levels, in aquatic systems (Richmond *et al.*, 2007). This link is essential for the optimal functioning of an ecosystem but at the same time it also implies a vulnerable point in a changing environment. Global changes in weather patterns driven by extreme events (floods and/or droughts) may have severe repercussion in reproduction, migration and abundances of copepods and thus in multiple species of aquatic food webs (Roff, 1992; Richmond *et al.*, 2007). Especially in a changing environment like an estuary, it is essential to understand the natural degree of variation of copepod communities.

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In general, copepods dominate mesozooplankton communities (Kršinić & Grbec, 2002; Cornils *et al.*, 2007). Among copepods, cyclopoids usually dominate the summer and autumn assemblages, whereas calanoids are predominant in winter and spring (Calbet *et al.*, 2001; Kršinić & Grbec, 2002; Cornils *et al.*, 2007). Indeed, copepod nauplii are most abundant in spring, followed by calanoid and cyclopoid copepodites, respectively. Whereas harpacticoids represent a less abundant fraction in copepod community (Calbet *et al.*, 2001; Kršinić & Grbec, 2002; Cornils *et al.*, 2007), this order constitutes a diverse group and the second most abundant with respect to meiofauna taxon (Huys & Boxshall, 1991; Suárez-Morales *et al.*, 2006). They are well adapted to different environments resulting in a wide range of morphological diversity in benthic and planktonic organisms (Huys & Boxshall, 1991). Harpacticoids are an important food source for higher trophic levels, whose reduction may cause profound impacts in many other estuarine species, such as fish communities, as well as in species of other habitats that spend their earlier life stages in estuaries. Although several studies demonstrated seasonal variations of these communities due to abiotic and biotic factors (e.g. temperature, salinity, food availability and turbidity), some species of copepods showed no clear seasonal trends (Calbet *et al.*, 2001; Kršinić & Grbec, 2002; Uriarte & Villate, 2004, 2005; Cornils *et al.*, 2007).

Despite several studies being based on the composition of marine harpacticoid fauna (Lang, 1948, 1965a; Huys *et al.*, 1996), they were restricted to a limited number of habitats or specific areas such as the Gulf of Mexico and other tropical areas (Fleeger & Clark, 1979; Rutledge & Fleeger, 1993; Fiers, 1995, 1996; Suárez-Morales *et al.*, 2000, 2006). In Portugal, few works have been carried out with a focus on harpacticoid copepods' ecology and taxonomy (Galhano, 1968; Noodt & Galhano, 1969; Morgado, 1997).

The objective of the present study is to document the natural variation of harpacticoid copepods in a typical European estuary. The Mondego estuary (shallow temperate mesotidal estuary) is located along the western coast of Portugal, a warm temperate region, constituting an important system to support human and industrial activities. Figueira da Foz city and its tourism activities cause seasonal pressure on the system (Marques *et al.*, 2002). Similarly to other estuaries, Mondego estuary receives large amounts of nutrients from agriculture fields, contributing to the developing of eutrophication events (Pardal *et al.*, 2000). So far, the Mondego estuary is documented in terms of the effects of eutrophication on benthic populations' structure and distribution (Pardal *et al.*, 2000; Cardoso *et al.*, 2004; Verdelhos *et al.*, 2005), and on zooplankton distribution and dynamics (Azeiteiro *et al.*, 1999; Gonçalves *et al.*, 2003; Marques *et al.*, 2006, 2007, 2008; Primo *et al.*, 2009). The first results of the implementation of the management plan to promote the recovery of Mondego estuary originated a reduction in the dissolved nutrients and green macroalgal blooms with the recovery of the seagrass beds (Verdelhos *et al.*, 2005; Dolbeth *et al.*, 2007; Cardoso *et al.*, 2008). Consequently, a higher biomass and growth production has been observed in the whole intertidal area. However, severe effects on macrobenthic assemblages due to naturally induced stressors started to be noticeable, with drastic socio-economic impacts related with high mortality of fish and the reduction of some economically important species of bivalves (e.g. *Scrobicularia plana* and *Cerastoderma edule*). Despite the extensive literature on the Mondego benthic and pelagic ecology, the species composition and distribution of harpacticoids in the two arms of the Mondego estuary remain unknown.

This paper presents the first study of Harpacticoida community and compares the distribution of harpacticoid species in both arms of the Mondego estuary in relation to spatial and seasonal environmental variations.

MATERIALS AND METHODS

Study site

The Mondego estuary, located on the western coast of Portugal (40°08′N 8°50′W) consists of two arms—north and south—with different hydrological characteristics, separated by Murraceira Island (Figure 1). The northern arm with a lower residence time (<1 day), is deeper (4–8 m at high tide, tidal range 1–3 m) and forms the main navigation channel, serving the Figueira da Foz harbour and connects directly to the Mondego River. The southern arm is more shallow (2–4 m deep, at high tide, tidal range about 1–3 m), with higher residence times (2–8 days). This channel is largely silted up, especially in the upstream areas, forcing most of the freshwater discharge to flow out through the northern arm (Pardal *et al.*, 2000; Cardoso *et al.*, 2004). The water circulation in the southern arm is predominantly due to the tidal cycle and to a relatively small freshwater input of a tributary, the Pranto River, which is artificially controlled by a sluice, according to the water needs on rice fields (Marques *et al.*, 2002). As for many other regions, this system suffers from direct and indirect human effects related to intensive agriculture, harbour facilities and economic growth at the regional scale. Since the late 1980s, the south arm shows symptoms of eutrophication as a result of anthropogenic activities. The system is gradually recovering after the implementation of mitigation measures in 1998 which enhanced environmental quality (Cardoso *et al.*, 2005; Dolbeth *et al.*, 2007). Until the beginning of 2006 this subsystem was almost silted up in the upstream areas. Since then, several works have been carried out in order to establish the connection between the two arms (Dolbeth *et al.*, 2007).

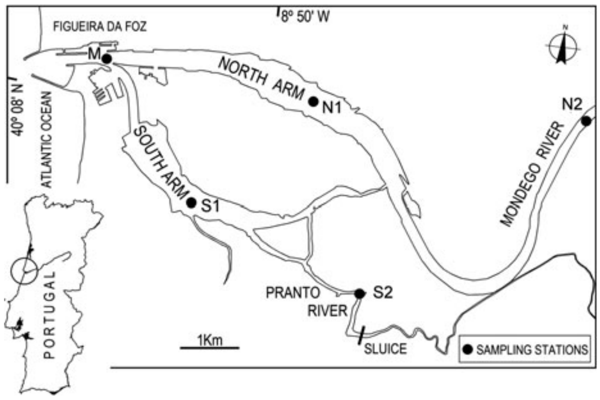

Fig. 1. Map of the Mondego estuary, located on the western coast of Portugal. Sampling stations are indicated: M, mouth station; N1 and N2, northern arm stations; S1 and S2, southern arm stations.

Sampling

Harpacticoid samples were collected monthly from February 2005 to January 2007 at five stations of Mondego estuary situated in both arms of the estuary in order to cover a maximum and representative area of the system (M, mouth of the estuary; N1 and N2, northern arm of the estuary; S1 and S2, southern arm of the estuary, Figure 1). Sampling was carried out during high tide, with a 63-μm mesh Bongo net, with 0.30 m diameter and the organisms were fixed in 4% buffered formaldehyde. Environmental parameters (salinity, temperature, dissolved oxygen concentration, pH and transparency) were measured *in situ*. In each sampling station subsurface water samples were collected for nutrient concentration analysis (Si, PO_4, NO_2, NO_3, NH_4), determination of chlorophyll-*a* (Chl-*a*) and total suspended solids (TSS). Monthly precipitation values were measured at the Soure 13 F/01G station and acquired from INAG—Portuguese Water Institute (http://snirh.inag.pt).

Laboratory procedures

Standard methods were followed to determine nutrient concentrations (Limnologisk Metodik, 1992—for ammonia and

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phosphate; Strickland & Parsons, 1972—for nitrate and nitrite). Chlorophyll-*a* concentration was determined by filtering 500–1000 ml of water through GF/C filters, which were then treated with acetone (90%) to extract the Chl-*a* measured at 630, 647, 665 and 750 nm (Parsons *et al.*, 1985). Dry weight (TSS) was estimated by filtering 500–1000 ml water through Whatman GF/C filters, tarred and dried at 60°C for 72 hours and combustion at 450°C for 8 hours (APHA, 1995).

From each sample, all harpacticoid copepods were counted, picked out and stored in ethanol. Glycerine slides were prepared with *in toto* adults. Harpacticoid copepods were identified to the lowest taxonomic level using the identification keys and reference books by Lang (1948, 1965a), Huys *et al.* (1996), Boxshall & Halsey (2004) and original genus and species descriptions. The nomenclature of families and genera in the checklist followed Bodin (1997) and Wells (2007).

Data analysis

A cluster analysis using the PRIMER statistical package (Clarke & Warwick, 2001) was performed in order to group harpacticoid species with similar distribution and occurrence. The Bray–Curtis similarity index was followed to calculate distance between groups, using complete linkage as clustering method. A redundancy data analysis (RDA) was carried out, using the CANOCO v. 4.5 package (ter Braak & Smilauer, 1998), on both matrices where columns were the taxonomic groups and environmental parameters, respectively, and rows of the seasonal data, which were estimated by averaging the monthly values from each station, to identify the relationship between species distribution and environmental factors. Prior to these analyses, Harpactioida densities were square-root transformed.

A spatial and seasonal variation analysis was performed for the five sampling stations in order to investigate and compare the seasonal variability of community structure. The seasonal periods defined are: winter—December, January and February; spring—March, April and May; summer—June, July and August; autumn—September, October and November. Each season account with two average months (e.g. Spring 05 and Spring 06) whereas the winter season reports to three average months (W05; W06 and W07).

RESULTS AND DISCUSSION

Environmental parameters

Annual precipitation average from 1961 to 1990 was 1016 mm (http://snirh.inag.pt). Figure 2 shows the normal climate for central Portugal (IM—Portuguese Weather Institute, http://web.meteo.pt). The study period presented different hydrological years: 2005–2006 more arid, and 2006–2007 a period with periodic wet months (Figure 2). Nevertheless, precipitation was lower (2005–2006: 855.9 mm and 2006–2007: 866.5 mm) than the normal precipitation average (1016 mm). In general, nitrate and silica concentrations showed a reduction in their values from winter to summer, but phosphate concentrations showed an irregular seasonal pattern, with higher values being associated with the upstream stations (Table 1). In the upstream stations an increase of Chl-*a* concentration was observed, especially in spring and summer at the north arm station, and for all seasons, except in spring, in the south arm station (e.g. Station N2: 10.48 in spring and 11.11 mg m^{-3} in summer; Station S2: 18.97 in winter and 17.33 in summer; and 14.69 mg m^{-3} in autumn). Total suspended solids (TSS) showed a decrease from the upstream towards the downstream stations in the northern arm, except in spring/summer, where the highest values were obtained at Station N1 (25.06 and 26.06 mg l^{-1}). In the southern arm, a decrease from upstream to downstream stations was also found. Turbidity values revealed a rise from downstream to upstream stations in both arms (Table 1). In general, the water temperature was higher in spring and summer and lower in autumn and winter, following a typical pattern for temperate latitudes. The values ranged from 10.46°C to 24.26°C, reaching minimum and maximum values during winter and summer at Station S2, respectively. The Stations S1 and M had the highest salinities, ranging from 15.32 psu (in winter at Station M) to 31.56 psu (in summer at Station S1). In general, Station N2 showed the

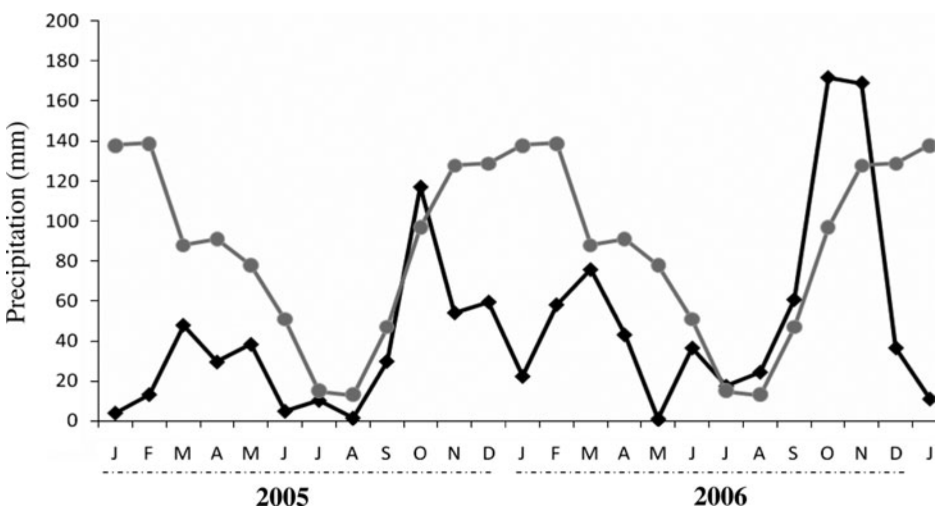

Fig. 2. Monthly precipitation (mm) in Mondego estuary during the study period (2005–2006 and 2006–2007). Grey filled circles represent a monthly average of 1961 to 1990 (http://snirh.inag.pt).

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Table 1. Physical and chemical parameters (Si, silica; PO_4, phosphates; NO_2, nitrites; NO_3, nitrates; NH_4, ammonia; Chl-*a*, chlorophyll-*a*; TSS, total suspended solids; O_2, dissolved oxygen; Temp, temperature; Sal, salinity; Turb, turbidity) at each sampling station ((M, mouth station; N1 and N2, northern arm stations, S1 and S2, southern arm stations), during seasonal cycles (W, winter (December, January and February); Sp, spring (March, April and May); S, summer (June, July and August); A, autumn (September, October and November)). Average values of two sampling actions in two years.

		Si (mg l^{-1})	PO_4 (mg l^{-1})	NO_2 (mg l^{-1})	NO_3 (mg l^{-1})	NH_4 (mg l^{-1})	Chl-*a* (mg m^{-3}	TSS (mg l^{-1})	pH	O_2 (mg l^{-1})	Temp (°C)	Sal (psu)	Turb (m)
M	**W**	1.80	0.03	0.01	0.80	0.05	2.75	15.76	7.91	10.60	11.63	15.32	1.30
	Sp	0.70	0.02	0.01	0.28	0.04	3.28	21.81	8.06	9.39	16.32	25.77	2.30
	S	0.66	0.03	0.00	0.33	0.06	3.95	22.93	7.94	8.30	18.20	28.74	2.74
	A	0.44	0.02	0.01	0.25	0.02	3.47	23.08	8.01	8.92	16.92	19.64	1.28
N1	**W**	2.05	0.04	0.01	1.00	0.11	4.02	14.41	8.09	10.21	11.45	11.68	0.97
	Sp	0.52	0.03	0.01	0.30	0.06	4.07	25.06	8.15	9.42	16.00	27.37	1.58
	S	0.53	0.02	0.00	0.12	0.03	3.00	26.06	7.59	8.07	19.32	24.50	1.90
	A	0.88	0.03	0.01	0.27	0.04	3.31	21.43	8.08	8.13	17.04	12.24	1.14
N2	**W**	2.54	0.04	0.03	1.58	0.16	6.80	6.47	8.04	9.97	10.51	0.93	0.84
	Sp	1.91	0.05	0.03	1.14	0.12	10.48	15.74	7.90	7.98	18.55	6.75	0.98
	S	1.28	0.05	0.01	0.49	0.06	11.11	19.00	7.34	6.59	23.54	10.76	1.02
	A	1.13	0.04	0.02	0.66	0.10	5.02	18.96	7.80	7.54	17.88	1.20	0.96
S1	**W**	1.33	0.03	0.01	0.58	0.05	2.66	19.21	7.95	10.21	12.01	20.81	1.12
	Sp	0.52	0.02	0.01	0.24	0.05	3.93	28.10	8.14	9.65	16.55	30.68	1.50
	S	0.43	0.02	0.01	0.15	0.04	3.92	26.79	7.84	8.10	18.54	31.56	1.52
	A	0.61	0.02	0.01	0.14	0.03	3.31	25.95	7.91	6.74	16.98	28.80	1.10
S2	**W**	1.93	0.05	0.04	0.65	0.24	18.97	25.84	7.69	9.01	10.46	13.31	0.57
	Sp	1.41	0.06	0.04	0.31	0.38	9.58	38.36	7.87	7.40	20.82	22.72	0.57
	S	1.50	0.06	0.03	0.07	0.35	17.33	32.07	7.54	6.06	24.26	19.22	0.50
	A	1.12	0.06	0.05	0.22	0.33	14.69	36.23	7.44	6.55	18.40	19.88	0.56

lowest values of salinity (winter 0.93, spring 6.75, summer 10.76 and autumn 1.20 psu).

Harpacticoid copepod composition

The harpacticoid copepods belonged to 14 harpacticoid families in which 13 species were identified plus six specimens that were identified to the genus level (namely *Canuella*, *Microsetella*, *Ectinosoma*, *Mesochra*, *Harpacticus* and *Parapseudoleptomesochra*). Table 2 gives a taxonomic overview of the species identified. The families Ectinosomatidae and Ameiridae were the most diverse followed by the other families represented by only one species/genus. *Euterpina acutifrons* (family Euterpinidae) and *Paronychocamptus nanus* (family Laophontidae) were widely distributed in the estuary. In spite of the initial aim of our sampling campaign to collect planktonic copepods, several harpacticoid copepods that were collected in the water column are benthic forms. A checklist of the harpacticoid copepods identified in Mondego estuary is provided below. Scientific names are employed for each taxonomic group to the lowest taxonomic level possible in order to give the most complete nomenclature information.

Subclass COPEPODA Milne-Edwards, 1840

Order HARPACTICOIDA Sars, 1903
Suborder POLYARTHRA Lang, 1944
Family CANUELLIDAE Brady, 1880a
Canuella sp. T. & A. Scott, 1893

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family EUTERPINIDAE Brian, 1921
Euterpina acutifrons Dana, 1848

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family ECTINOSOMATIDAE Sars, 1903
Microsetella norvegica Boeck, 1865
Microsetella sp. Brady & Roberston, 1873
Ectinosoma melaniceps Boeck, 1865
Ectinosoma sp. Boeck, 1865

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family MIRACIIDAE Dana, 1846
Macrosetella gracilis Dana, 1847

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family CANTHOCAMPTIDAE Sars, 1906
Mesochra sp. Boeck, 1865

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family METIDAE Sars, 1910
Metis cfr. *ignea* Philippi, 1843

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family TACHIDIIDAE Sars, 1909
Tachidius discipes Giesbrecht, 1881

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family HUNTEMANNIDAE Por, 1986
Nannopus palustris Brady, 1880

Table 2. List of abbreviations and individual abundance (%) of Harpacticoid species (subclass Copepoda Milne-Edwards, 1840; order Harpacticoida Sars, 1903) used in data analyses.

Suborder	Family	Taxa	Abbreviation	Abundance (%)
Polyarthra Lang, 1944	Canuellidae Brady, 1880a	*Canuella* sp. T. & A. Scott, 1893	Can	0.02
	Canthocamptidae Sars, 1906	*Mesochra* sp. Boeck, 1865	Me	0.06
	Metidae Sars, 1910	*Metis* cfr. *ignea* Philippi, 1843	Metig	0.06
	Tachidiidae Sars, 1909	*Tachidius discipes* Giesbrecht, 1881	Tadi	0.03
		Copepodite *Tachidius discipes*	CpTadi	0.10
	Ectinosomatidae Sars, 1903	*Ectinosoma melaniceps* Boeck, 1865	Ectmel	0.02
		Ectinosoma sp. Boeck, 1865	Ect	0.21
		Copepodite *Ectinosoma* sp.	CpEct	0.02
		Microsetella norvegica Boeck, 1865	Mino	0.67
		Copepodite *Microsetella norvegica*	CpMino	0.20
		Microsetella sp. Brady & Robertson, 1873	Misp	1.21
		Copepodite *Microsetella* sp.	CpMisp	1.20
	Huntemannidae Por, 1986	*Nannopus palustris* Brady, 1880	Napa	0.17
	Harpacticidae Sars, 1904	*Harpacticus obscurus* T. Scott, 1895	Harob	0.07
		Harpacticus sp. Milne-Edwards, 1840	Harsp	0.02
	Darcythompsonidae Lang, 1936	*Leptocaris brevicornis* van Douwe, 1904	Lepbr	0.02
	Leptastacidae Lang, 1948	*Paraleptastacus* cfr. *spinicauda* T. & A. Scott, 1895	Parspi	2.12
	Ameiridae Monard, 1927	*Praeleptomesochra phreatica* Pesce, 1981a	Praeph	0.02
Oligoarthra Lang, 1944		*Parapseudoleptomesochra* sp. Lang, 1965b	Paraps	0.13
	Phyllognathopodidae Gurney, 1932	*Phyllognathopus viguieri* (Maupas, 1892)	Phylvi	0.03
	Miraciidae Dana, 1846	*Macrosetella gracilis* Dana, 1847	Macgr	0.05
		Copepodite *Macrosetella gracilis*	CpMacgr	0.02
	Euterpinidae Brian, 1921	*Euterpina acutifrons* Dana, 1848	Euac	21.45
		Copepodite *Euterpina acutifrons*	CpEuac	34.57
	Laophontidae T. Scott, 1905	*Paronychocamptus nanus* Sars, 1980	Pana	20.16
		Copepodite *Paronychocamptus nanus*	CpPana	13.88

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family HARPACTICIDAE Sars, 1904
Harpacticus obscurus T. Scott, 1895
Harpacticus sp. Milne-Edwards, 1840

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family DARCYTHOMPSONIDAE Lang, 1936
Leptocaris brevicornis van Douwe, 1904

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family LEPTASTACIDAE Lang, 1948
Paraleptastacus cfr. *spinicauda* T. & A. Scott, 1895

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family AMEIRIDAE Monard, 1927
Praeleptomesochra phreatica Pesce, 1981a
Parapseudoleptomesochra sp. Lang, 1965b

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family LAOPHONTIDAE T. Scott, 1905
Paronychocamptus nanus Sars, 1980

Order HARPACTICOIDA Sars, 1903
Suborder OLIGOARTHRA Lang, 1944
Family PHYLLOGNATHOPODIDAE Gurney, 1932
Phyllognathopus viguieri (Maupas, 1892)

This species list is comparable to the one reported by Morgado (1997) from the estuary of Ria de Aveiro, located 50 km north of the Mondego estuary. In spite of the fact that Morgado (1997) used a 125-μm sieve, several harpacticoid species were found in common, namely adults and copepodites of *Euterpina acutifrons*, *Microsetella* sp., *Ectinosoma* sp., *Harpacticus obscurus*, *Tachidius discipes*, *Metis* cfr. *ignea*, *Mesochra* sp. and *Paronychocamptus nanus*. Morgado (1997) found *T. discipes* and *E. acutifrons* to be the two most abundant species.

A cluster analysis (Figure 3) showed the affinity between species/genus identified in the five stations of the Mondego estuary. Group I is composed of the most related species, *Metis* cfr. *ignea* and *Tachidius discipes*, due to their high distribution in Station S2 during the winter of 2005. The high abundance of *T. discipes* in Station S2 is related to the highest TSS value that was recorded in this southern arm station, in comparison with the other sampling sites (see Table 1). Group II is the most representative, comprising the species with the highest densities and highest frequency of occurrence in the estuary. The distribution of this group of harpacticoid species may be due to their high level of adaptability to environmental variance. Species of Groups II, III, V, VI and VII presented a high affinity due to high densities recorded in autumn 2005, spring 2006, spring 2005/2006, winter 2005 and 2007 and autumn 2006, respectively. Group IV comprised rare species, occurring at one or two of the sampling stations, in a specific season: *Canuella* sp., *Phyllognathopus viguieri*, *Microsetella norvegica* (juveniles) and *Macrosetella gracilis* (juveniles) occurred mostly in winter in the northern arm stations; *Microsetella norvegica* (juveniles) and *Ectinosoma melaniceps* appeared in summer in the south and north arm stations, respectively; *Leptocaris brevicornis* appeared in autumn in the southern arm of the estuary. The species of this group still showed no affinity among them nor between the species of the other groups.

A RDA analysis (Figure 4) shows the occurrence of harpacticoid species in relation to the environmental variables (NO_3, PO_4, NH_4, Si, NO_2, pH, turbidity, Chl-*a*, temperature, O_2, salinity and TSS). The eigenvalues for axis 1 (0.361) explained 91.6% of the cumulative variance in the species data relative to the total variation explained by the environmental variables. Adults and juveniles of *Microsetella* sp., and *Euterpina acutifrons* are associated with maximal concentrations of NO_2 and TSS, while *Canuella* sp., *Praeleptomesochra phreatica*, *Nannopus palustris*, *Macrosetella gracilis* and *Parapseudoleptomesochra* sp. correlate with higher amounts of Si and PO_4. Similarly, Pesce (1981) reported *P. phreatica* from freshwater samples from North Africa (Morocco). *Ectinosoma melaniceps*, *Tachidius discipes* (including juveniles), *Metis* cfr. *ignea*, *Harpacticus obscurus* and *Mesochra* sp. grouped in the lower part of the plot, associated with high salinities and O_2 concentrations. The harpacticoids—*Paronychocamptus nanus* (adults and juveniles), *Leptocaris brevicornis* and *Paraleptastacus spinicauda*—were found in the upper part of the plot, associated with high concentrations of NH_4.

A seasonal distribution of harpacticoid species (adults and juveniles) was performed to compare species' densities in the sampling stations and to define the most representative species in each sampling station and season. The northern arm of the estuary especially was characterized by a high density of harpacticoids during the whole year, with the highest abundances in Station N2 (Figure 5).

More harpacticoids (copepodites and adults) were found in the northern and southern arms (Figures 6 & 7). Copepodites and adults of *Euterpina acutifrons* and *Paronychocampus nanus* were dominant depending on the season. In general, *P. nanus* dominated in winter (W) and spring (Sp) seasons, except in spring at Stations M, N1 and S1, which were dominated by *E. acutifrons*. *Paraleptastacus* cfr. *spinicauda* showed an occurrence of 5–10% at all stations, except for Station M, where no distribution was observed. *Microstella* sp. showed a similar percentage of occurrences in spring/summer at Stations M and S1 and in winter/summer at Station S2. In general, this genus appears in the five stations sampled in

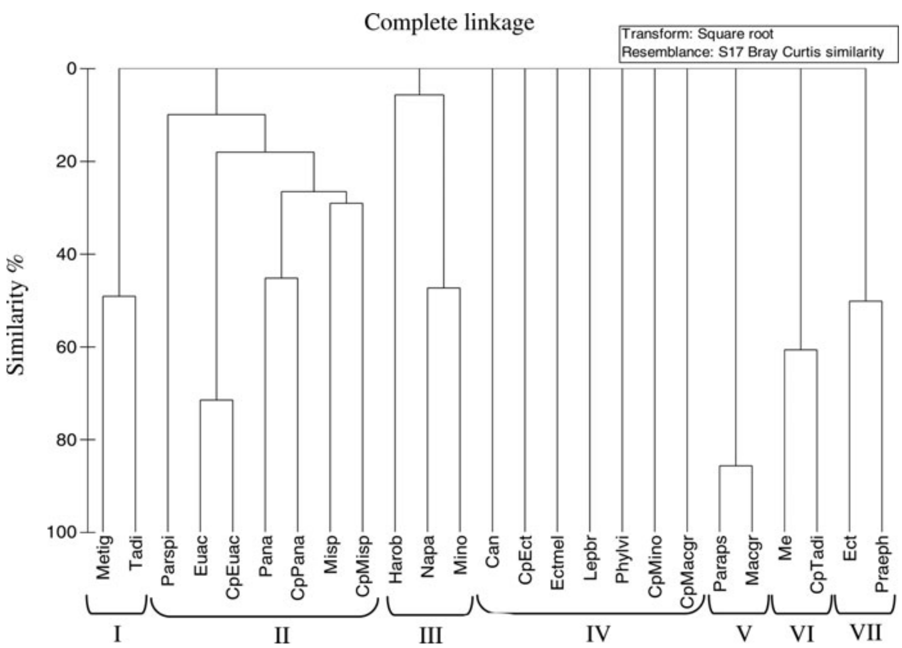

Fig. 3. Cladogram (cluster analysis) grouping harpacticoid species–genus groups based on their distribution in different stations. The seven species–groups of Harpacticoida are indicated by roman numbers.

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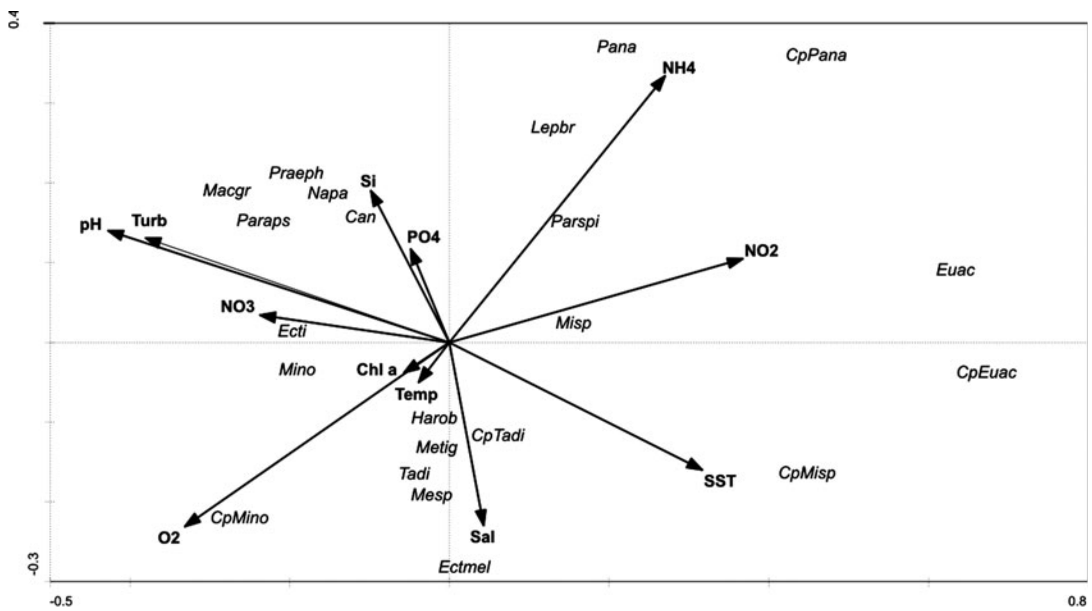

Fig. 4. Results of RDA analysis. Relationships between harpacticoid species distribution and environmental variables in Mondego estuary from February 2005 to January 2007.

the estuary during spring/summer, but showed lower abundances at Stations N1 and N2. These results are reinforced in Figure 8, which compares the seasonal and spatial occurrence of the two most abundant species in the estuary (*E. acutifrons* and *P. nanus*) with neritic–oceanic species (*Microsetella* sp. and *Macrosetella gracilis*). *Microsetella* sp. and *Macrosetella gracilis* represented a lower abundance. Still, the last one appears more regularly in the southern arm than in the northern arm, with a maximum density of *Microsetella* sp. in the five sampling stations.

Harpacticoid copepods are known to occur in the sediment (benthic) and on aquatic plants (epiphytic). However, the presence of benthic harpacticoids in plankton samples can be due to suspension processes and also to active migration (Walters & Bell, 1994; Villate, 1997; Uriarte & Villate, 2005). The deepness of the sampling sites is not below 2 m, achieving 8 m in the north arm. In addition, the sampling always occurred at high tide, when the tidal range was at maximum, as stated above.

Uriarte & Villate (2004, 2005) studied the abundance and spatial distribution of copepods species and other zooplankton species in the polluted estuary of Bilbao and the unperturbed Urdaibai estuary of the Basque coast (Bay of Biscay). The authors stated that mainly environmental factors affect the distribution and structure of copepods and other zooplankton communities. In addition, copepods showed a low tolerance to hypoxia as they are present in higher abundance in unpolluted areas or in areas with moderate pollution (Siokou-Fragou & Papathanassiou, 1991; Roman *et al.*, 1993). In general, inter-estuarine comparisons showed that copepod abundances were higher in the Urdaibai estuary at high salinities, with *E. acutrifrons* being abundant at all salinities and meiobenthic harpacticoids only at the stations with salinities

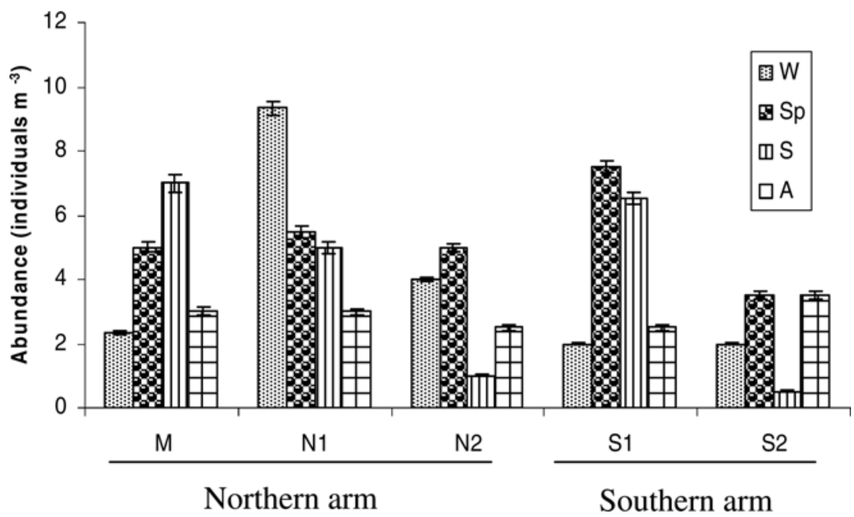

Fig. 5. Average harpacticoid copepod densities (±standard error) in the different seasons and different stations: M, mouth station; N1 and N2, northern arm stations; S1 and S2, southern arm stations.

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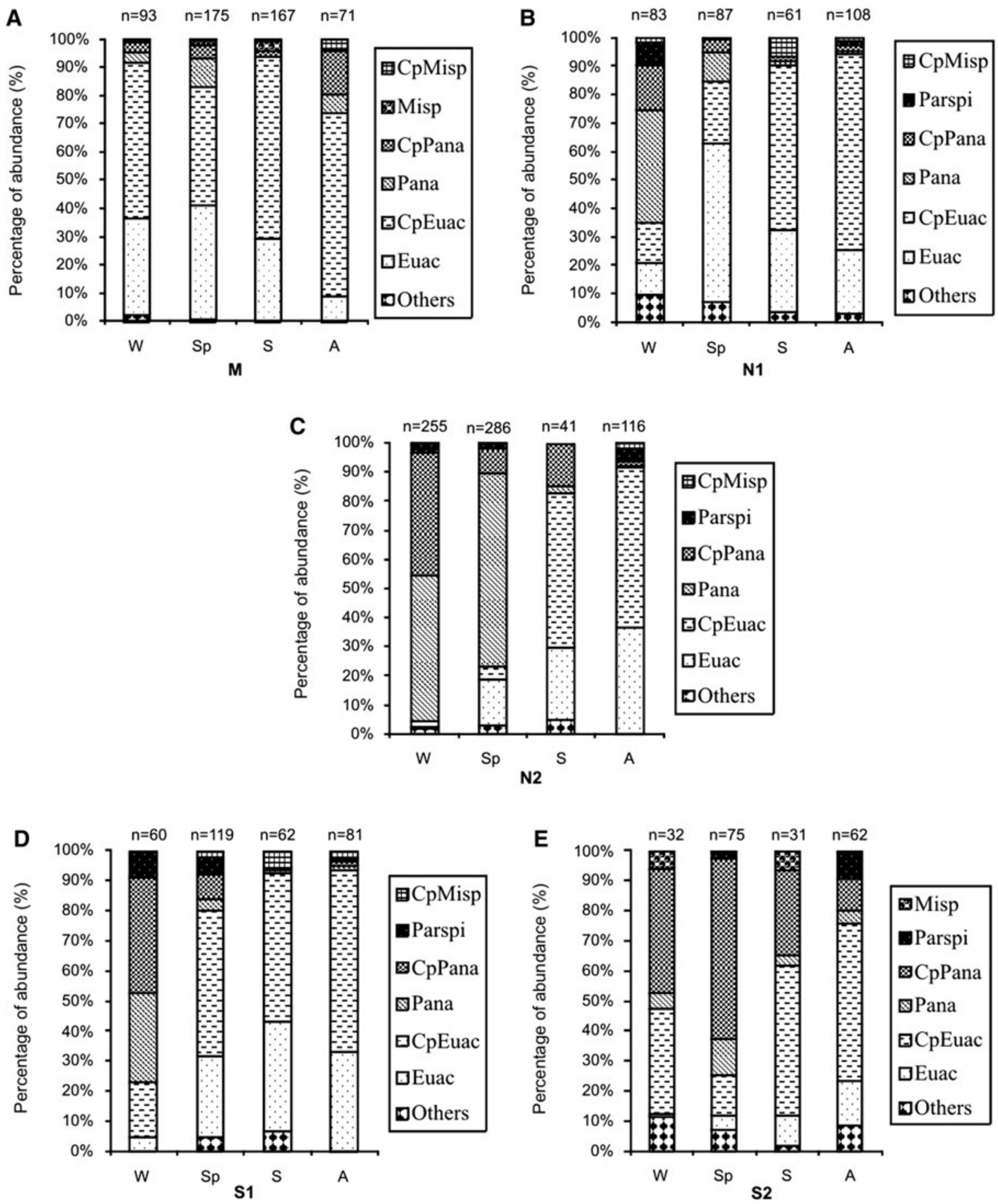

Fig. 6. Relative abundance (%) of harpacticoid species/genera in each sampling station. (A) Mouth station; (B) Station N1; (C) Station N2; (D) Station S1; (E) Station S2, during seasonal period: W, winter; Sp, spring; S, summer; A, autumn.

of 31 and 34 psu. Some copepod species show clear seasonal patterns of dominance or differences between estuaries or stations. For instance, *Paracalanus parvus* and *Acartia clausi* had high abundance in the Urdaibai and Bilbao estuaries, respectively, and were the copepod species with the highest seasonal alternation in the dominance in each estuary and between estuaries' differences. *Oncaea media* and *Temora stylifera* still dominated in summer with no significant differences between estuaries or salinity stations, while *Pseudocalanus elongates in full please* showed higher abundance in winter/spring and in sites with low salinities. As reported by other authors, harpacticoids were found in small densities, showing no clear seasonal trends and differing between estuaries rather than between salinity stations (Uriarte & Villate, 2005). *Euterpina acutifrons* was much more abundant in the unpolluted estuary of Urdaibai presenting a uniform distribution in both estuaries. This species revealed a high adaptation to environmental factors and also to pollution gradients.

Kršinić & Grbec (2002) studied the distribution of small zooplankton at two stations in the Otranto Strait (eastern

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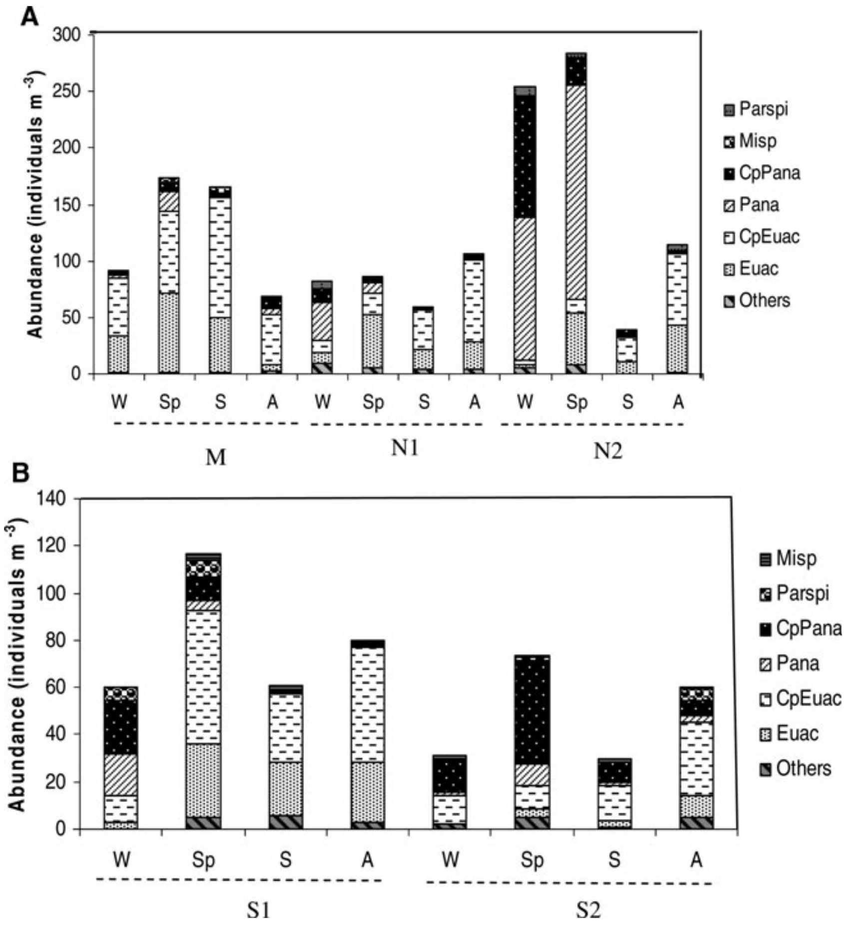

Fig. 7. Seasonal pattern of the composition of harpacticoid copepods community in (A) northern and (B) southern arms of Mondego estuary.

Mediterranean), with a plankton net of 53-μm mesh size. Although harpacticoids were the group with the lowest density, they dominated in spring (May 1990). However, the authors did not observe, unlike other groups, any seasonal variation of small copepod fauna in the study area. Copepodites and adults of *Microsetella* sp. and *Macrosetella* sp. were identified, whereas other taxa were rare or absent. Similarly, our results show higher abundance in spring, for all stations, although, the species showed no significant variance in the sampling period. Accordingly, *Microsetella* sp. was one of the species found with the highest abundance in Mondego estuary. In the northern Gulf of Aqaba (Red Sea), Cornils *et al.* (2007) studied the seasonal cycle of mesozooplankton using a net of 200-μm mesh size and found harpacticoids occurring in their lowest numbers (less than 1%) with the highest densities of copepods in spring and in autumn. Other studies have been carried out by Calbet *et al.* (2001) in the Bay of Blanes (north-west Mediterranean) within an annual cycle. Sampling included an oblique tow using a 200-μm net and vertical tows using a 53-μm net. Despite copepods' groups found by other authors, Calbet *et al.* (2001) concluded cyclopoids copepods dominated the summer and autumn communities while calanoid copepods were predominant in winter and spring, with no reference to harpacticoids found.

So far, there are few reports on harpacticoid copepod species in Portuguese estuarine systems. The harpacticoid families identified in the present study are benthic forms which may live in and on the fine and upper sandy sediments, which explains their presence in zooplankton samplings. The low deepness of the study sites and tidal influence in eroding the sediment bed and resuspension mechanisms creates new sedimentation areas colonized by harpacticoid copepods. Morgado (1997) observed a comparable composition and distribution pattern of harpacticoid species, collected with a 125-μm plankton net in the shallow Ria de Aveiro estuary (western Portugal). The wide variety of harpacticoid forms exploits different and particular types of habitats (sediment, phytal and planktonic), which require specialized features (Bell *et al.*, 1987; Boxshall & Halsey, 2004; Suárez-Morales *et al.*, 2006). In some cases, these copepods are associated with other organisms (e.g. polychaeta) since they can benefit from the fact that: (1) the harpacticoids are less subjected to predation or disturbance, since the structure confer a source of predation refuge; and (2) the organism (the structure builder) may stimulate microbial growth providing food availability (Thistle & Eckman, 1988). Accordingly, species exhibit a variety of morphological characteristics and may be found migrating from different habitats. Different forms may still be represented in the Mondego estuary fauna.

Our study is a first attempt to give an overview of the harpacticoid species present in the Mondego estuary, a temperate shallow estuarine system from southern Europe. A next step forward could be to analyse the function of these harpacticoids in the system. Since harpacticoid copepods form an important link between primary production and higher trophic levels (De Troch *et al.*, 1998, 2005; Buffan-Dubau & Carman,

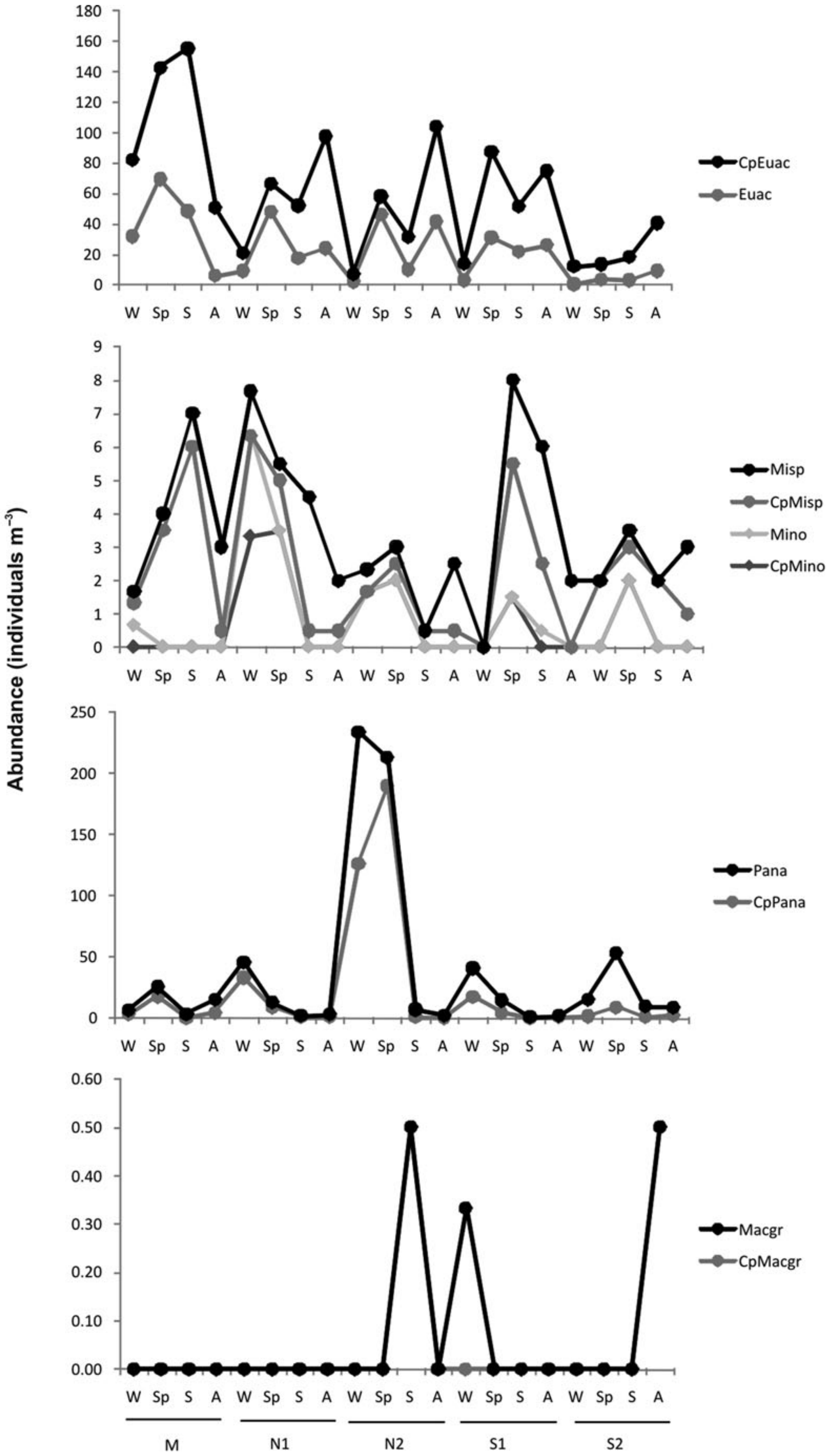

Fig. 8. Seasonal cycle of the most abundant harpacticoid copepods species (*Euterpina acutifrons* and *Paronychocamptus nanus*) versus neritic–oceanic species (*Macrosetella gracilis* and *Microsetella norvegica*). February 2005–January 2007.

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2000), unravelling their role in Mondego estuary will be an essential contribution to understand the functioning of ecosystems and the effects of potential threats.

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