Responses of Copepoda Life-History Stages to Climatic Variability in a Southern-European Temperate Estuary

Ana M.M. Gonçalves[1,*], Miguel Â. Pardal[1], Sónia C. Marques[1], Susana Mendes[2,3], María J. Fernández-Gómez[3], María P. Galindo-Villardón[3], and Ulisses M. Azeiteiro[1,4]

[1]*Centre for Functional Ecology (CFE), Department of Life Sciences, University of Coimbra, Apartado 3046,3001-401 Coimbra, Portugal*
[2]*GIRM-Marine Resources Research Group, School of Tourism and Maritime Technology, Polytechnic Institute of Leiria, Campus 4, 2520-641 Peniche, Portugal*
[3]*Department of Statistics, University of Salamanca,37007 Salamanca, Spain*
[4]*Department of Sciences and Technology, Universidade Aberta, 4200-055Porto, Portugal*

Ana M.M. Gonçalves, Miguel Â. Pardal, Sónia C. Marques, Susana Mendes, María J. Fernández-Gómez, María P. Galindo-Villardón, and Ulisses M. Azeiteiro (2012) Responses of Copepoda life-history stages to climatic variability in a southern-European temperate estuary. *Zoological Studies* **51**(3): 321-335. In this study, we investigated the effects of an extreme climate event (severe drought) on copepod ecology. Monthly sampling events were conducted from 2005 to 2007, at 5 stations, using 63- and 335-μm-mesh nets in Mondego Estuary, Portugal. The Calanoida were represented mainly by *Acartia clausi*, *Temora longicornis*, and *A. tonsa* and the Cyclopoida by *Oithona plumifera* and *Acanthocyclops robustus*. *Acartia clausi* and *T. longicornis* dominated at the mouth and middle estuary; *A. tonsa* and *A. robustus* were associated with the upper estuary while *O. plumifera* showed the highest densities in the downstream section. Nauplii occurred in higher densities at the mouth. Relationships of copepod assemblages and environmental factors were analyzed using the STATICO method which allowed us to distinguish the combination of factors that most contributed to these relationships. Winter was characterized by high concentrations of nutrients, cold waters, and low salinities, while summer was generally characterized by high values of phosphate, salinity, and temperature. Marine and estuarine species (mainly copepodites) showed high densities in summer. Freshwater species occurred at maximal densities in winter, coincidently with higher river flow. Copepod assemblages showed a clear seasonal pattern that was superimposed on the interannual variability. Moreover, a severe drought was responsible for the dominance of marine species. http://zoolstud.sinica.edu.tw/Journals/51.3/321.pdf

Key words: Copepods, Life stages, Mondego Estuary, Seasonal and interannual variability, STATICO.

Estuaries are transition zones between rivers and the sea and differ in biotic (e.g., predation and competition) and abiotic conditions (e.g., temperature, salinity, and food quantity and quality). Plankton (and mainly zooplankton) are known to be particularly sensitive to such changes because they are strongly influenced by climatic features and variations in hydrological conditions (Ara 2001, Isari et al. 2007, Hwang et al. 2010a b, Kâ and Hwang 2011). Several studies focused on zooplankton ecology and dynamics (Uriarte and Villate 2005, Lam-Hoai et al. 2006, Hafferssas and Seridji 2010, Hwang et al. 2010b), with some specifically examining the Mondego Estuary (Azeiteiro et al. 2000, Morgado et al. 2007, Marques et al. 2009, Primo et al. 2009, Gonçalves et al. 2010a b). Copepods are usually the dominant group of mesozooplankton and play important roles in trophic food webs since they are a link between primary producers and secondary

consumers (Richmond et al. 2007, Hwang et al. 2010b, Hsiao et al. 2011, Kâ and Hwang 2011). Still, studies simultaneously integrating copepod life-history stages (nauplii, juveniles, and adults) with the aim of examining their ecology over contrasting environmental conditions are scarce in the literature mainly in southern European systems (Kršinić et al. 2007). Studies were developed for either a single species or taxonomic category (Incze and Ainaire 1994, Hansen et al. 2004) in a spatiotemporal distribution perspective, or laboratory tests were performed to investigate organisms' responses to environmental factors (Cook et al. 2007). Moreover, earlier life-history stages (mainly naupliar stages) are pivotal for the subsequent development and growth of juveniles and for the maintenance of copepod populations (Cook et al. 2007). Temperature, and food quality and quantity are the main environmental factors controlling stage durations of copepods (Morgado et al. 2007, Hafferssas and Seridji 2010), while preferential salinity regimes for egg production are chosen in order to obtain the highest hatching success (Chinnery and Williams 2004, Cook et al. 2007). Thus, studies that do not address early copepod stages have no information on changes in species dynamics and thus cannot infer data about seasonal fluctuations due to a lack of information. This frequent under-sampling of small copepod species, and mainly earlier stages (Dias et al. 2010, Hwang et al. 2010a), may lead to a limited view of the ecology of planktonic systems (particularly in estuarine areas). However, few studies focused on the impacts of large-scale weather events, such as an extreme drought, on copepod communities, in order to produce a holistic and integrative view of an ecosystem's responses to global climate change. Such changes associated with long-term biological series may contribute to further knowledge about interannual variations in abundance and diversity of copepod communities.

The aims of this study were to focus on the life-history stages (nauplii, juveniles, and adults) of copepods under 2 contrasting environmental conditions (an extremely dry year (2005) and regular years (2006 and 2007) in order to: (1) determine the major environmental parameters explaining copepod dynamics in a southern-European shallow temperate estuary, (2) reveal seasonal variations in distribution patterns of copepods in response to hydrological factors, and (3) determine interannual variations of copepod assemblages during an extreme drought.

MATERIALS AND METHODS

Study area and sampling sites

Mondego Estuary is a small mesotidal system of 8.6 km^2, located on the western Atlantic coast of Portugal (40°08'N, 8°50'W) (Fig. 1). It comprises 2 channels, north and south, separated by the island of Murraceira which is about 7 km from the shore, and the channels join again near the mouth. These 2 arms present different hydrological characteristics. The northern arm is deeper (4-8 m during high tide, with a tidal range of about 2-3 m), has a low residence time (< 1 d), and is the location of a commercial harbor and the main navigation channel. At neap tides, this arm is characterized by a salt-wedge during low tide, which changes to a partially mixed water column at high tide. At spring tides, it is characterized by a partially mixed water column at low tide and a well mixed one at high tide (Cunha and Dinis 2002). The southern arm is shallower (2-4 m deep during high tide) and has higher residence times (2-8 d), and the water circulation is mostly dependent on tides and on freshwater input from a small tributary system, the Pranto River. Freshwater discharge of this river is controlled by a sluice according to the water needs of Mondego valley rice fields.

Sample collection and laboratory procedures

Copepod samples were collected monthly in Mondego Estuary from Feb. 2005 to Dec. 2007, during high tide, at 5 sampling stations (M, N1, N2, S1, and S2) distributed throughout both arms (Fig. 1). Copepods were collected by subsurface tows with a 335-μm-mesh Bongo net (with a diameter of 0.5 m) (COMARTEC, LDA - Portugal) and a 63-μm-mesh net (with a diameter of 0.30 m), equipped with a Hydro-Bios flow-meter (COMARTEC, LDA - Portugal) in the mouth (to estimate the volume of water filtered by the nets). Samples were fixed and preserved in 4% buffered formaldehyde in seawater. In order to determine the number of taxa and abundances, all samples were counted (individuals (ind.)/m^3) and identified to the lowest taxonomic level possible. Water samples were collected at each sampling station to determine nutrient concentrations (Strickland and Parsons 1972 for nitrate and nitrite, mg/L; Limnologisk Metodik 1992 for phosphate and ammonia, mg/L), chlorophyll (chl)-*a* concentration (Parsons et al. 1985, mg/m^3), and total suspended solids (TSS; APHA 1995, mg/L).

Additionally, several hydrological parameters were measured *in situ*: water temperature (°C) and salinity (WTW Cond 330i, WTW Wissenschaftlich - Technischewerkstätten, Germany), dissolved oxygen (DO) (WTW OXI 330i, mg/L), pH (WTW pH 330i), and transparency as Secchi-disc depth (m). Monthly precipitation and long-term monthly average precipitation (1971-2000) were measured at the Soure 13 F/01G station and acquired from INAG, the Portuguese Water Institute (http://snirh. inag.pt). Freshwater runoff from the Mondego River was obtained from INAG station Açude Ponte Coimbra 12G/01AE, near the city of Coimbra (located 40 km upstream).

Data analysis

Only the most abundant taxa with a minimal mean occurrence of 0.1% of the total density observed in the study area were considered. This cutoff eliminated species that occurred rarely, as some were only observed on few or rare occasions. Moreover, well-represented species can be viewed as proxies of copepod dynamics and ecosystem functioning.

In order to investigate the spatial variability in the copepod community structure, the species density and environmental parameters of each sampling site were combined to generate 2 series of tables (Fig. 2): one for environmental variables and the other for species densities. Each pair of tables contains information on the same sampling period (spring 2005 to autumn 2007) for 5 sites (M, N1, N2, S1, and S2). Species abundance was transformed using a $\log(x + 1)$ function prior to the calculations, to minimize the dominating effect of exceptional catches. Environmental data were normalized by scaling to a unit standard deviation within the mode to homogenize the table.

The common structure between the environmental and species-density tables and the stability of this structure across the sampling stations were assessed by the STATICO method (Simier et al. 1999, Thioulouse et al. 2004). The STATICO method was used by Simier et al. (2006) and Carassou and Ponton (2007) to study spatial and seasonal variabilities of fish assemblages in a Gambian estuary and coastal areas of New Caledonia, respectively, and by Mendes et al. (2009) to describe the spatiotemporal structure of diatom assemblages in Ria de Aveiro (Portugal). This method proceeds in 3 stages. (1) In the 1st stage, each table is analyzed by a 1-table method (a normed principal component analysis (PCA) of
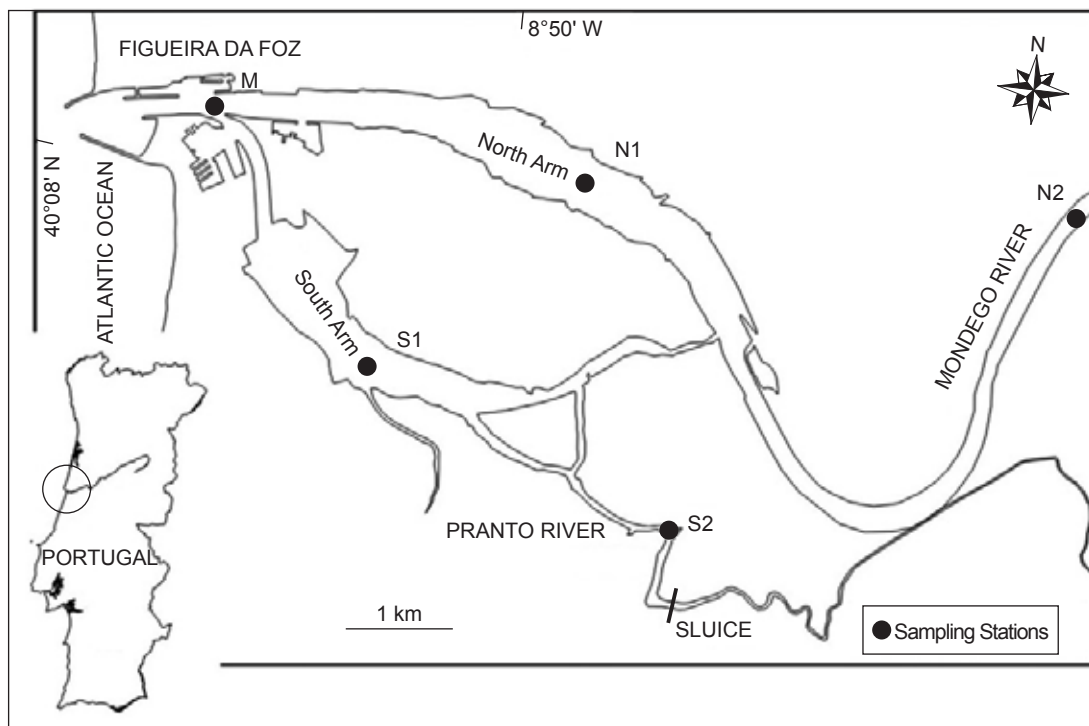

Fig. 1. Location of Mondego Estuary on the western coast of Portugal and the 5 sampling stations within the estuary (M, mouth station; N1 and N2, stations of the north arm; S1 and S2, stations of the south arm).

environmental variables and a centered PCA of species data). A vectorial correlation (RV) matrix between stations (in terms of the co-structure between environment and species density) is calculated, which allows comparisons of stations and a representation of the proximity between stations. The function of this step is to attribute a weight to each station's sub-matrix. (2) Each pair of tables is linked by a co-inertia analysis (Dolédec and Chessel 1994) which provides an average image of the co-structure (species-variables). (3) A partial triadic analysis (Thioulouse and Chessel 1987) is then used to analyze this sequence. It is a 3-step procedure, namely interstructure, compromise, and intrastructure (or trajectory) analyses. In fact, the compromise is the main step of the analysis (Thioulouse et al. 2004). It is based on a compromise table that is computed as the weighted mean of all tables of the series, using the components of the 1st eigenvector of the interstructure as weights.

This table is called the compromise, and it has the same dimensions and the same structure and meaning as the tables of the series. It is analyzed by a PCA, and gives a picture of structures common to all tables. STATICO also enables one to plot the projection of the sampling seasons of each original table on the compromise axes (of the PCA factor map), in terms of the structures of species abundances and environmental factors. Hence, it is possible to discuss correlations between species distributions and environmental factors. Calculations and graphs were done using ADE-4 software (Thioulouse et al. 1997).
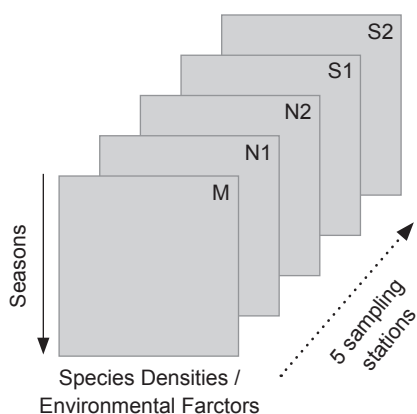

Fig. 2. Three-way data structure of environmental parameters and species densities at 5 sampling stations.

RESULTS

Climate: precipitation and environmental background

In Mondego Estuary, clear seasonal and yearly variations in rainfall and freshwater discharge were observed during the 3-yr period (Fig. 3A). In 2005, an extreme drought was recorded with precipitation and freshwater discharge values much lower than the 1971-2000 average, causing as severe a drought as any recorded in the 20th century in Portugal. A severe reduction in freshwater flow was evidenced by the lowest value in 2005 at almost 48-fold lower than the highest value recorded in 2006. In 2006 and 2007, precipitation values were closer to the average except in Oct. 2006 when above-mean precipitation was registered (Fig. 3A). So, the last 2 yr of the study period were considered regular years.

In general, water temperature and salinity showed similar variation patterns during the study period with lower values in winter months (Fig. 3B). Nevertheless, salinity was highly variable during the sampling period, with the lowest value in 2006 being almost 14-fold lower than the highest in 2005 (corresponding to the extreme drought). As expected, the highest values of salinity were observed in 2005. Water temperature showed a typical pattern for temperate regions, ranging 10.12 ± 0.51 to 20.86 ± 2.55°C (Fig. 3B).

Analysis of the interstructure (between-station analysis)

From the 55 different copepod species identified, 28 (including naupliar, copepodite, and adult stages) were dominant and regularly occurred. The interstructure factor map of the STATICO analysis, based on 12 environmental variables (pH, DO, temperature, salinity, transparency, chl-*a*, TSS, and nutrients) and on abundances of the 28 copepod species from different stages (nauplii, copepodites, and adults), showed that the relationship between environmental variables and species appeared to be stronger in N1 (with the longest arrow), followed in decreasing order of importance in the compromise, by M, S1, N2, and S2. This means that the compromise was more strongly influenced by N1, M, and S1 (Fig. 4A). The remaining sampling sites (N2 and S2) presented short arrows, meaning that the corresponding tables

were less structured and had lower importance in the compromise. The 1st 2 axes respectively represented 69% and 11% of the total variability (Fig. 4B).

The matrix presenting the RV between the station sub-matrices (Table 1) showed the strongest correlation (RV = 0.77) between stations S1 and N1, and the weakest one (RV = 0.32) between stations S2 and M. From the interstructure analysis, it is possible to analyze the

contribution of each sub-matrix in constructing the compromise (Table 1). The contribution represents the weight of each sub-matrix in defining the compromise. It seems that sub-matrices M, N1, and S1 contributed a larger part to defining the compromise, which suggests that the other stations had more-particular structures that led to weaker weights. Regarding $\cos^2$ (Table 1), an indicator of how much the compromise expresses the information contained in each table, station
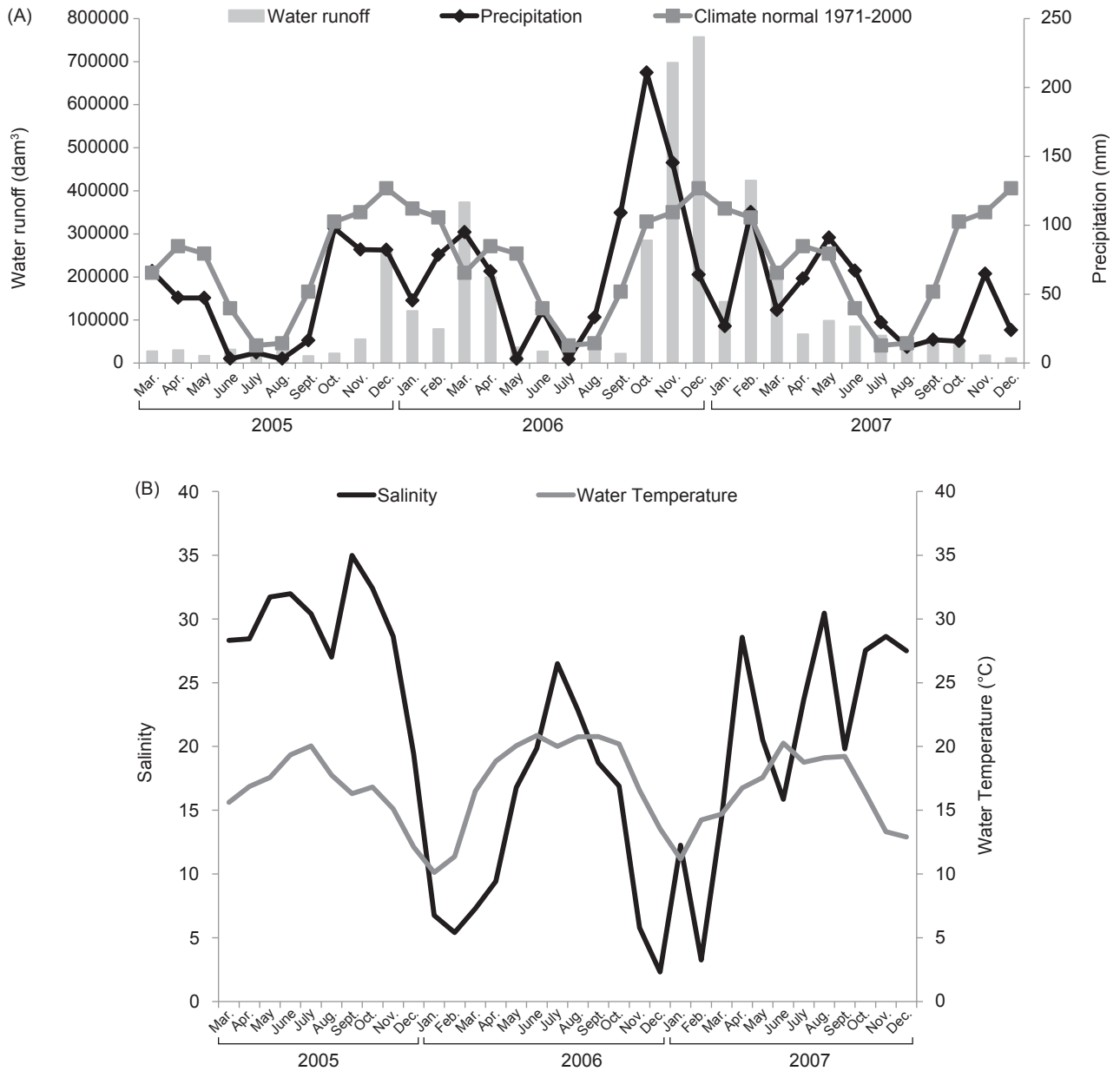

Fig. 3. (A) Monthly precipitation (mm) in Mondego Estuary during the study period (2005-2007). Gray filled squares represent monthly averages of 1971-2000 (http://snirh.inag.pt) and (B) monthly averages of salinity (psu) and water temperature (°C) during the study period (2005-2006 and 2006-2007).

N1 was the one that fit the best ($cos^2 = 0.81$), followed by stations M and S1 ($cos^2 = 0.71$ and 0.64, respectively). Lastly, seasonal dynamics at stations N2 and S2 showed the least accuracy with the compromise ($cos^2 = 0.54$ and 0.24, respectively), in terms of the co-structure between environment factors and species densities.

Spatial structure

Factor plots of the 1st 2 axes of the compromise analysis are shown for the copepod community and environmental variables (Fig. 5). The 1st axis was clearly dominant, and accounted
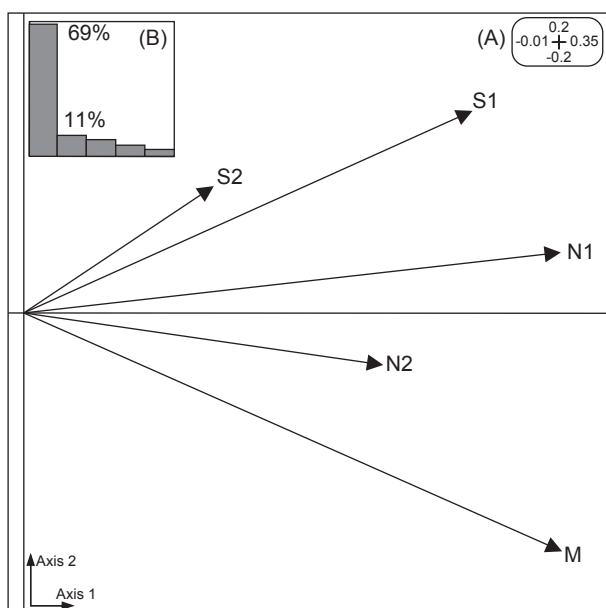
for 89% of the explained variance, in contrast to the 2nd axis which accounted for only 4% of the explained variance and was thus much less significant (Fig. 5C). Therefore, these axes provided a good summary and typology of the species' spatial organization, on the basis of a common structure, for sampling sites across the 3 yr.

The factor map of the compromise for the copepod community in the STATICO analysis indicated that the most abundant species in the samples were mainly associated with the same environmental variables: temperature and salinity (Fig. 5A, B). Copepodites of *Oithona plumifera* Baird, 1843 *Euterpina acutifrons* Dana, 1848 copepodites of *E. acutifrons*, copepodites of *Oithona* sp., and copepodites of *Temora longicornis* O.F. Müller, 1785 were more abundant when salinity and transparency were high and the concentrations of ammonium, nitrite, nitrate, and silica were low (Fig. 5A, B). In particular, copepodites of *O. plumifera*, adults and copepodites of *E. acutifrons* were clearly influenced by high values of temperature, with respective abundances during the study period of $2.62 \times 10^4 \pm 1.33 \times 10^5$, $3.92 \times 10^3 \pm 1.44 \times 10^4$, and $9.59 \times 10^3 \pm 4.99 \times 10^4$ ind./m^3. Beyond that, the high density of copepodites of *O. plumifera* ($2.62 \times 10^4 \pm 1.33 \times 10^5$ ind./m^3) was also under the influence of high phosphate values and low DO concentrations. In contrast, were copepodites and adults of *Paronychocamptus nanus* Sars, 1980 ($1.10 \times 10^3 \pm 5.75 \times 10^3$ and $3.59 \times 10^2 \pm 1.52 \times 10^3$ ind./m^3 respectively), copepodites and adults of *Acanthocyclops robustus* G.O. Sars, 1863 ($1.67 \times 10^3 \pm 1.12 \times 10^4$ and 1.20 ± 3.76 ind./m^3, respectively), and copepodites of *Centropages* sp. ($6.46 \times 10^2 \pm 5.48 \times 10^3$ ind./m^3). These were more abundant in waters with lower salinities and temperatures combined with reduced transparency

Fig. 4. Interstructure factor map of the STATICO analysis of Mondego Estuary data. (A) This map shows the importance of each sampling station in the compromise (M, mouth station; N1 and N2, stations of the north arm; S1 and S2, stations of the south arm). The scales are given in the boxes.

Table 1. Typological value indices. RV, correlation matrix which contains the cosines between the tables; Weights, weights of the tables in the compromise; Cos^2, squared cosine between the table and approximated compromise

Sampling site	RV					Weights	Cos^2
M	1					0.55	0.71
N1	0.72	1				0.55	0.81
N2	0.60	0.65	1			0.37	0.54
S1	0.59	0.77	0.49	1		0.46	0.64
S2	0.32	0.41	0.43	0.44	1	0.19	0.24

Sampling sites are defined in figure 1.

and TSS values. Moreover, copepodites of *Acartia* sp. ($1.94 \times 10^3 \pm 7.97 \times 10^3$ ind./m³), *Clausocalanus* sp. ($2.19 \times 10^2 \pm 1.17 \times 10^3$ ind./m³), *Acartia clausi* Giesbrecht, 1889 ($9.94 \times 10^2 \pm 3.66 \times 10^3$ ind./m³), *T. longicornis* ($1.05 \times 10^3 \pm 5.09 \times 10^3$ ind./m³), *Oithona* sp. ($1.46 \times 10^4 \pm 5.00 \times 10^4$ ind./m³), and *Paracalanus*-type form (calanoid) ($2.22 \times 10^3 \pm 8.85 \times 10^3$ ind./m³) were abundant when salinity and TSS concentrations were elevated and nitrite, ammonium, pH, and phosphate were low (Fig. 5A, B). Copepodites of *Pseudocalanus* sp., *Paracalanus* sp., and *Clausocalanus* sp. are referred to as the *Paracalanus*-type form when they do not show the main characteristics of each genus for their identification. Regardless of the species, the 1st axis of the compromise factor map for environmental parameters mainly described negative associations of salinity with nitrate and silica (Fig. 5B). On this factorial plan, pH, ammonium, and chl-*a* concentrations were weakly represented (Fig. 5B). The 2nd axis showed negative associations with phosphate, temperature, and DO.

Internal typology of each site in the composition of species and variations of environmental factors

For each survey, the projection on the com-

promise axes of the 28 species (see Table 2 for species codes) and 12 environmental variables is shown in figure 6. The stable part of the species-environment dynamics revealed by the compromise analysis was better expressed by surveys at sampling stations M, N1, and S1 (Fig. 6). The N2 and S2 surveys also showed similar species-environment dynamics, however with a slight difference in the density of copepodites of *A. clausi*, *T. longicornis*, *Acartia* sp., *Clausocalanus* sp., and the *Paracalanus*-type form combined with the dynamics of environmental factors at those stations (Fig. 6). Species' distribution patterns were more similar among downstream stations, showing high correlations of species abundances with environmental factors. At upstream stations, species presented lower abundances and lower correlations with environmental factors, mainly at the southern station (S2). On a spatial scale, salinity and temperature were unrelated at downstream stations, whereas at upstream stations, these 2 factors were positively correlated (Fig. 6B). Furthermore, nutrient concentrations appeared positively correlated on the left side of axis 1, along which the freshwater species *A. robustus* was distributed. Marine species were mostly located on the right-hand side of the 1st axis, with higher temperatures, salinities, and TSS concentrations. Moreover, by examining certain
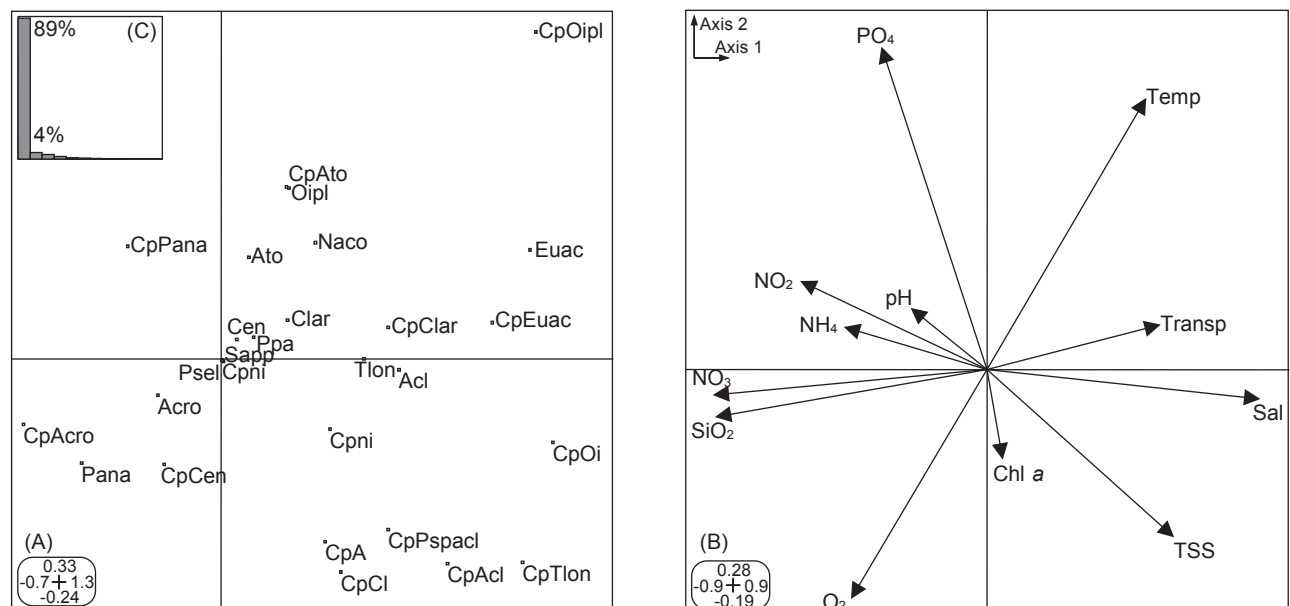

Fig. 5. Compromise factor map of the STATICO analysis of copepod species variables (A) and environmental parameters (B). (C) Eigenvalue diagram. Scales of the axes are given in the boxes. Chl-*a*, chlorophyll-*a*; Temp, water temperature; Sal, salinity; O₂, dissolved oxygen; TSS, total suspended solids; Transp, transparency; PO₄, phosphate; NO₂, nitrite; NO₃, nitrate; SiO₂, silica; NH₄, ammonia. See table 2 for species codes.

Table 2. List of species (including life stages) and their abbreviations (Abbr.) used in the multivariate analysis (STATICO). Average copepod densities (individuals/m^3) recorded during the study period are presented

Taxa		Abbr.	Habitat	Average (range)
	Nauplii Copepoda	Naco		3.57×10^5 (0-5.61×10^7)
	Copepodite unidentified	Cpni		2.60×10^3 (0-4.09×10^5)
Calanoida				
Acartiidae	*Copepodite Acartia* sp.	CpA		1.94×10^3 (0-3.04×10^5)
	Copepodite Acartia clausi	CpAcl	Marine	9.94×10^2 (0-1.56×10^5)
	Acartia clausi	Acl	Marine	84.5 (0-1.33×10^4)
	Copepodite Acartia tonsa	CpAto	Estuarine	4.30×10^3 (0-6.74×10^5)
	Acartia tonsa	Ato	Estuarine	1.93×10^2 (0-3.02×10^4)
Temoridae	*Copepodite Temora longicornis*	CpTelo	Marine	1.05×10^3 (0-1.64×10^5)
	Temora longicornis	Telo	Marine	78.4 (0-1.23×10^4)
Centropagidae	*Copepodite Centropages* sp.	CpCen		6.46×10^2 (0-1.01×10^5)
	Centropages sp.	Cen		9.58×10^{-1} (0-1.50×10^2)
Clausocalanidae	*Copepodite Clausocalanus* sp.	CpCl		2.19×10^2 (0-3.43×10^4)
	Copepodite *Clausocalanus arcuicornis*	CpClar	Marine	5.65×10^2 (0-8.88×10^4)
	Clausocalanus arcuicornis	Clar	Marine	7.68 (0-1.21×10^3)
	Pseudocalanus elongatus	Psel	Marine	3.11×10^{-2} (0-4.88)
Paracalanidae	*Paracalanus parvus*	Ppa	Marine	66.4 (0-1.04×10^4)
	Copepodite of *Paracalanus*-type form	CpPspacl		2.22×10^3 (0-3.49×10^5)
Cyclopoida				
Oithonidae	Copepodite *Oithona* sp.	CpOi		1.46×10^4 (0-2.30×10^6)
	Copepodite *Oithona plumifera*	CpOipl	Marine	2.62×10^4 (0-4.12×10^6)
	Oithona plumifera	Oipl	Marine	4.10×10^2 (0-6.44×10^4)
Cyclopidae	Copepodite *Acanthocyclops robustus*	CpAcro	Freshwater	1.67×10^3 (0-2.62×10^5)
	Acanthocyclops robustus	Acro	Freshwater	1.20 (0-1.89×10^2)
Harpacticoida				
Euterpinidae	Copepodite *Euterpina acutifrons*	CpEuac	Estuarine	9.59×10^3 (0-1.51×10^6)
	Euterpina acutifrons	Euac	Estuarine	3.92×10^3 (0-6.16×10^5)
Laophontidae	Copepodite *Paronychocamptus nanus*	CpPana	Marine	1.10×10^3 (0-1.73×10^5)
	Paronychocamptus nanus	Pana	Marine	3.59×10^2 (0-5.63×10^4)
Poecilostomatoida				
Sapphirinidae	*Sapphirina* sp.	Sapp		9.10×10^{-3} (0-1.43)

environmental characteristics, it was confirmed that stations M, N1, S1 were mutually more similar, compared to the other ones.

The major groups of copepods, calanoids, cyclopoids, and harpacticoids, were represented in the study area. Calanoids were mainly represented by marine species (e.g., *A. clausi* and *T. longicornis*) and by the estuarine species *A. tonsa* Dana, 1849. Cyclopoids were mainly represented by the euryhaline species, *O. plumifera*, and the freshwater species, *A. robustus. Acartia clausi* and *T. longicornis* principally dominated at the mouth (M) and middle estuary (N1 and S1), while *A. tonsa* and *A. robustus* were more highly associated with the upper estuary (N2 and S2). The cyclopoid, *O. plumifera*, mostly its copepodite stage, occurred along the estuary,

showing the highest densities at the mouth (M) and middle northern arm (N1). This species (mainly its juvenile stages) was one of the most abundant copepod species. Harpacticoid species were represented by the estuarine species, *E. acutifrons* and *P. nanus*, which always presented lower densities than Euterpinidae species. *Euterpina acutifrons* occurred along the entire salinity gradient, showing a wide distribution in the estuary. Although *P. nanus* was also found at all 5 sampling stations, this species showed higher densities in the middle estuary (N1 and S1). The order Poecilostomatoida was represented by *Sapphirina* sp. This taxon showed the lowest abundance of all the copepods ($9.10 \times 10^{-3} \pm 4.47 \times 10^{-2}$ ind./m^3) and was considered a rare species. Nauplii of copepods occurred along the entire estuary, but
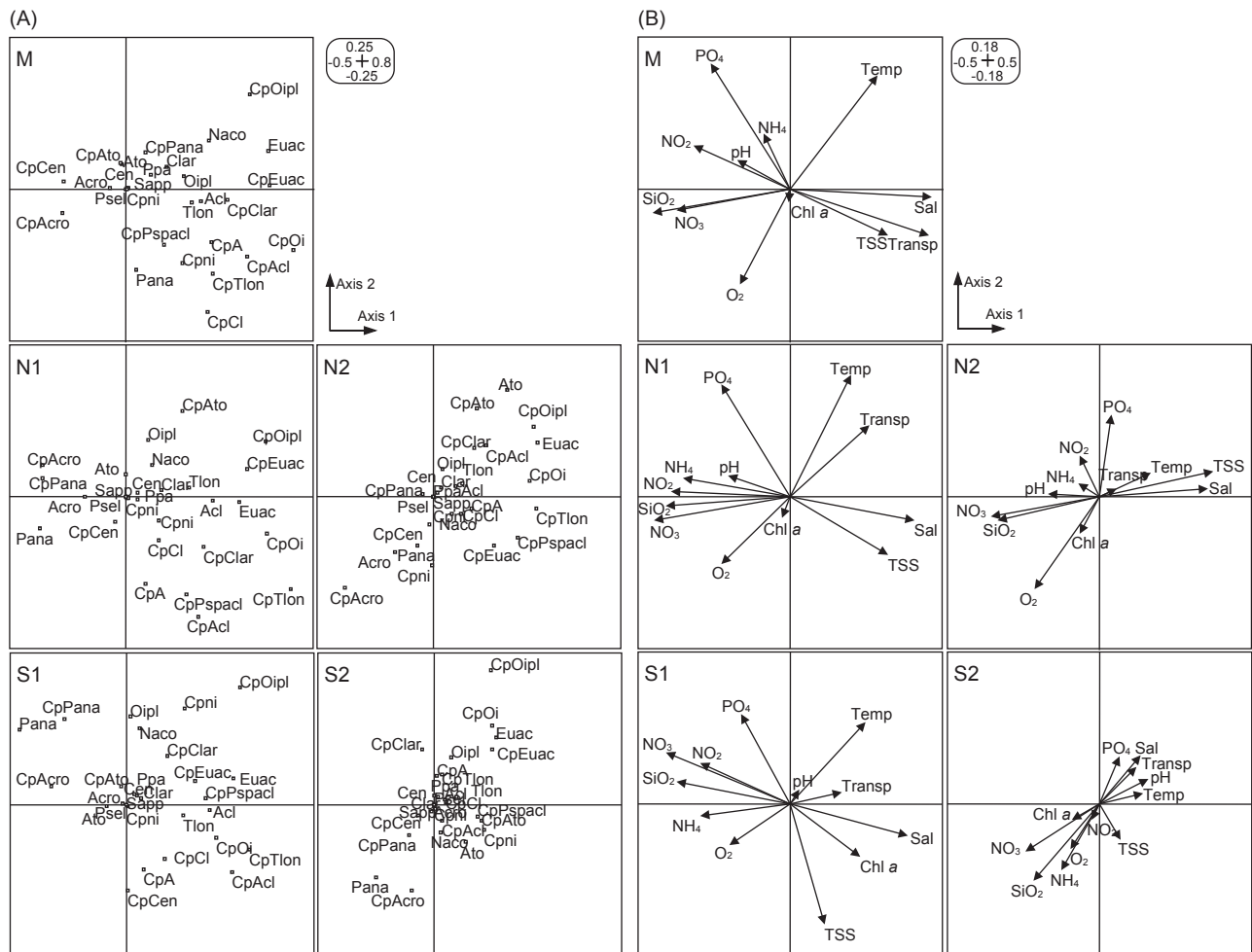

Fig. 6. Trajectory factor maps of the STATICO analysis. (A) Projection of the average position of copepod species at each sampling station and (B) projection of environmental variables at each sampling station (M, mouth station; N1 and N2, stations of the north arm; S1 and S2, stations of the south arm). Scales of the axes are given in the boxes. For species codes see table 2. See figure 5 for codes of environmental variables.

were observed at higher densities at the mouth station (M).

The co-structure graphics (divided according to sampling sites) clearly showed the dynamics of species-environment relationships and highlighted differences between seasons (Fig. 7). Projections of species points (origins of the arrows) were

located in different places according to the site from which they were dispersed from upstream stations (N2 and S2) to the mouth station (M). These positions were essentially related to salinity and temperature, with the smallest values observed at upstream stations. Regardless of the date, species points (origins of arrows) were
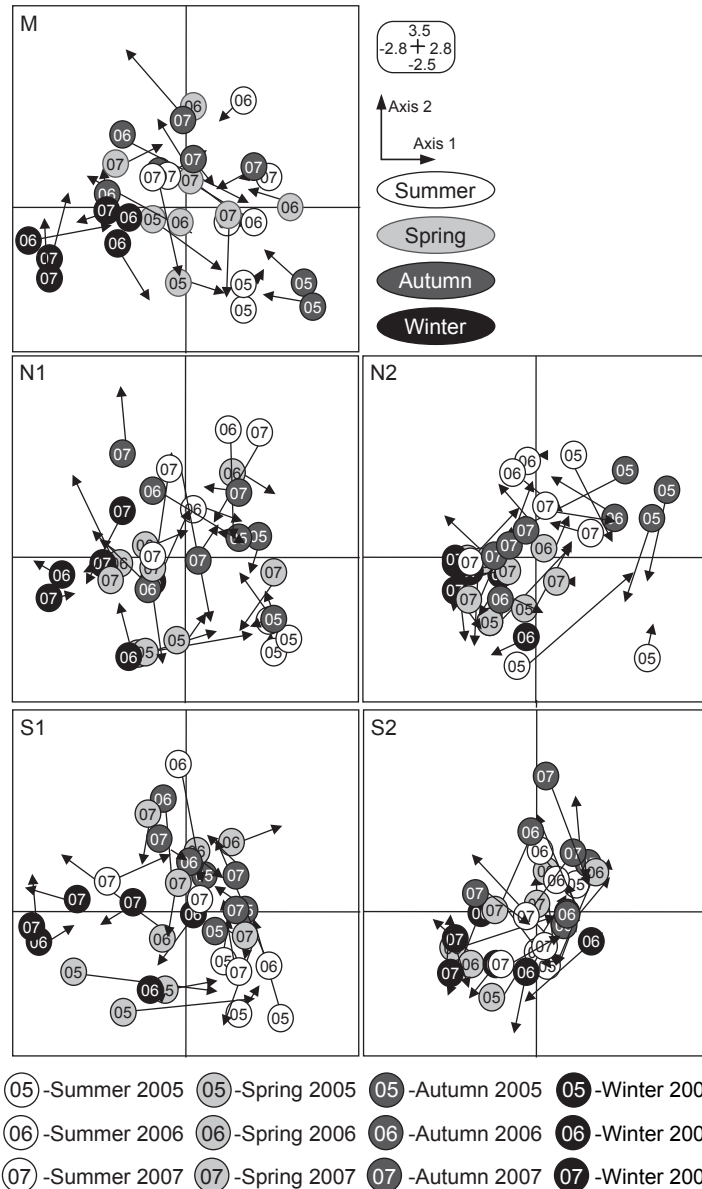

Fig. 7. Trajectory factor plots of the STATICO analysis. Projection of the seasons along the study period (2005-2007) in terms of both environmental factors and copepod structures. Graphs are given for each sampling site (M, mouth station; N1 and N2, stations of the north arm; S1 and S2, stations of the south arm) that showed the highest contribution to the co-structure between environmental factors and copepod abundances. Each sample is represented by 2 points: one is a projection of the row of the species table (circle: origin of an arrow), and the other is a projection of the row of the environmental table (end of an arrow). The length of the connecting line reveals the disagreement or the consensus between the 2 profiles (species/environment), i.e., the length of the line is proportional to the divergence between the datasets. When datasets agree very strongly, the arrow is short. Likewise, a long arrow demonstrates a locally weak relationship between environmental factors and copepod features for that case. Scales of the axes are given in the boxes.

more stable than environmental points, because they expressed the steady establishment of species assemblages in the estuary, despite the high environmental variability (principally of salinity, temperature, and phosphate). In general, winter was mostly projected on the left-hand side of the 1st axis, and was characterized by high concentrations of nitrate, nitrite, silica, and ammonium, in contrast to high salinity and warm water, and for the most part, with high densities of juveniles and adults of *A. robustus* and *P. nanus*. In contrast, nauplii of copepods appeared to be numerically less important during winter months. This season presented the most regular projection, and exceptions only occurred at S1 and S2. Summer was characterized by factors that described axis 2, which means that at stations M, N1, S1, and N2, summer mainly presented high values of phosphate, salinity, temperature, TSS, and DO. On the other hand, at S2, summer was influenced by the highest values of phosphate, transparency, salinity, pH, and temperature. From a species point of view, summer was principally characterized by high densities of copepodites of *O. plumifera*, *E. acutifrons* (adults and juveniles), copepodites of *Oithona* sp., copepodites of *A. clausi*, copepodites of *Acartia* sp., copepodites of *Paracalanus*-type form, copepodites of *T. longicornis*, and copepodites of *Clausocalanus* sp. Spring and autumn presented a mixed pattern, without a clearly dominant axis. Exceptions were at S1 in autumn (which mostly projected on the right-hand side of the 1st axis) and N2 in spring (which mostly projected on the left-hand side of the 1st axis). Arrows were mostly short in summer, expressing a good coincidence between environmental conditions and plankton structure. An exception occurred at N2, with a poor fit between copepod species' densities and environmental factors (long arrows) during some surveys in summer 2005. However, in this season, there was a higher correlation between the distribution of copepod densities and environmental factors under the direct influence of high values of phosphate, salinity, TSS, DO, and temperature at downstream stations (M, N1, and S1). Furthermore, there was a strong co-structure between species' densities and environmental factors at the upstream station in the southern arm (S2) under the influence of high values of phosphate, salinity, transparency, temperature, and pH, in summer months. On the other hand, at S1, all winter surveys presented short arrows expressing a strong co-structure

between species' densities and environmental factors, which means that environmental factors (high values of nitrate, nitrite, silica, and ammonia and low values of temperature and salinity) well explained the distribution of species in this season. At the upstream stations (S2 and N2), arrows were mostly long, which means lower correlations between the distribution of copepod densities and environmental factors.

DISCUSSION

This study focused on abundances of key copepod species and their respective life stages in Mondego Estuary, Portugal. Moreover, relationships between environmental factors and species' densities and distributions were also examined over a time course of 3 yr. Indeed, in Mondego Estuary, as in other coastal areas (Uriarte and Villate 2005, Hwang et al. 2010b, Kâ and Hwang 2011), the zooplankton group of the Copepoda was dominant. The most abundant copepod species (e.g., *A. clausi*, *T. longicornis*, *O. plumifera*, *A. tonsa*, *A. robustus*, *P. nanus*, and *E. acutifrons*) were associated with different environmental variables. The marine component of the community was associated with saline waters, high values of TSS, and low concentrations of nutrients, while the freshwater component was characterized by low salinity and high DO and nutrient concentrations.

Concerning seasonal variations, winter was marked by a higher presence of freshwater species and lower naupliar densities. Such abundance and distributional patterns were also observed in other works (Tackx et al. 2004, Primo et al. 2009, Gonçalves et al. 2010a b). In spring/summer months, there were higher abundances of *A. clausi*, *T. longicornis*, and *Clausocalanus arcuicornis* Dana, 1849 (mainly juveniles), while in summer/autumn months, copepodites of *O. plumifera* were more abundant. Similarly, Villate et al. (2004) reported higher abundances of the calanoids *A. clausi*, *Paracalanus parvus* Claus, 1863, *Clausocalanus* spp., *Pseudocalanus elongatus* Boeck, 1865, and *T. longicornis*, and small marine cyclopoids of the genera *Oithona* and *Oncaea*, which were correlated with saline waters, in estuarine systems of the Basque coast. Moreover, *Centropages typicus* Krøyer, 1849, *Oithona nana* Giesbrecht, 1892, and *P. parvus* are typically surface species with affinities for high temperature and low salinities, while *O. plumifera*,

Clausocalanus sp., *P. elongatus*, and *T. longicornis* were reported as species that prefer deeper waters with lower temperatures and higher salinities. Although several authors (Villate et al. 1993, Holste and Peck 2006) stated that copepod nauplii distribute towards higher salinities, naupliar stages show a high sensitivity to environmental factors. However, this behavior may change from species to species and also from nauplii to juveniles and adults of the same species, with some being more sensitive than others (Chinnery and Williams 2004). According to Incze and Ainaire (1994), the peak occurrence of copepod nauplii is in late spring and is related to the greatest concentrations of chl-*a*. Chinnery and Williams (2004) stated that differential distributions of different life stages in estuarine systems may be due to the poor swimming ability of naupliar stages. The weak ability to swim leads nauplii into areas of higher salinities and sweeps them towards the mouth of the estuary, where they stay and develop to later stages and afterwards move back up the estuary to assume their adult distribution patterns.

In Mondego Estuary, freshwater species showed higher densities at upstream stations (N2 and S2) because of the high influence of fresh water from the Mondego and Pranto Rivers. Marine and estuarine species were found at the middle stations (N1 and S1) due to the intrusion of marine water at both stations, while at the mouth station (M), a higher number of marine species was verified (Primo et al. 2009, Gonçalves et al. 2010b).

Downstream stations (M, S1, and N1) exhibited a strong correlation together with the most abundant species which were mainly associated with salinity and temperature and presented a rich marine fraction. This fact agrees with other studies carried out in a wide range of European estuaries (Mouny and Dauvin 2002, Tackx et al. 2004), and emphasizes the significant influence that salinity has on zooplankton abundance, composition, and distribution. The higher salinities (and lower freshwater inflow) registered in 2005 (the Portuguese Weather Institute, http://web.meteo.pt/clima.jsp, classified the drought period of 2005 as the most severe drought of the past 60 yr) led to an increase of marine zooplankters in the estuary. The same was reported by Primo et al. (2009) for mesozooplankton and by Martinho et al. (2007) for fish species. The severe drought in 2005 was responsible for a clear spatial shift in the copepod community, mostly dominated by marine species, which remained in subsequent regular climatic years (2006 and 2007). Preferences for marine conditions by naupliar forms led to a clear absence of an interannual distributional pattern in copepod assemblages. Seasonality was also detected in the study period, and drove the variability in zooplankton assemblages in Mondego Estuary. This study also contributed to further detailed knowledge of naupliar and copepodite stages, and also determined their spatial distribution patterns. In this study area, *A. tonsa* and *A. clausi* were copepods with higher abundances in Mondego Estuary and contributed to the increase in copepod dominance in the estuary. The former exhibited higher densities at upstream stations (N2 and S2), and the latter appeared in much higher densities at downstream stations (M, N1, and S1) (Primo et al. 2009, Gonçalves et al. 2010b). At estuaries of Bilbao (a polluted estuary) and Urdaibai (an undisturbed estuary), located on the Basque coast (Bay of Biscay) with salinities of 31-35 psu, Uriarte and Villate (2005) stated that responses of copepods may be related to water desalination and their tolerance to pollution. Those authors referred to *A. clausi* and *P. parvus* as the most abundant species in the Bilbao and Urdaibai estuaries. Still, at the latter estuary, higher densities of *P. elongatus*, *E. acutifrons*, and harpacticoids were also found. A significant reduction in copepod species with decreasing salinity was observed at the polluted estuary, while *E. acutifrons* and *Oithona helgolandica* Claus, 1863 did not show significant differences, which is in agreement with their widespread distributions. Similar to our results, some authors (Ara 2001, Uriarte and Villate 2005) stated that *E. acutifrons* did not show a clear seasonal trend, occurring throughout the year, and tolerating wide ranges of salinities and temperatures. The species' highest abundance is in estuaries and coastal waters rather than offshore waters (Björnberg 1963). Uriarte and Villate (2005) reported reductions of *A. clausi*, *P. parvus*, and *O. plumifera* with decreasing salinity, with the former species reaching higher abundances in spring. In addition, the distribution of *A. clausi* is affected by the presence of other species, such as *Acartia bifilosa* Giesbrecht, 1881, which does not survive very well at higher salinities as can *A. clausi*. Moreover, *A. clausi* showed higher hatching success at a salinity of 33.3 psu than did *A. bifilosa* (Chinnery and Williams 2004, Uriarte and Villate 2005). Indeed, *A. tonsa* seems to have physiological plasticity in terms of egg hatching, being the most tolerant of the *Acartia* congeners, with 50% hatching success at a salinity of 9 psu

(Holste and Peck 2006). In terms of distribution, *A. tonsa* is a common species in estuaries and European seas, occurring from temperate to subtropical waters; but its distribution is restricted to habitats with abundant food (David et al. 2007, Morgado et al. 2007). Chinnery and Williams (2004) classified *A. tonsa* and *A. clausi* as summer species. David et al. (2007) stated that *A. tonsa* shows a seasonal pattern in northern European estuaries characterized by a peak abundance in late summer and autumn, with a 2nd spring peak observed in southern European estuaries. Two assumptions could explain these peaks in abundance: 1. warmer conditions, as temperature is known to be a major factor controlling the biological cycle of copepods (Gaudy 1972, Hsiao et al. 2011) and the world distribution of *A. tonsa* (Conover 1956); and 2. diapause/subitanious eggs, that hatch when the water temperature exceeds 10°C, and then the population rapidly increases above 15°C (McAlice 1981), which might explain the spring peak and high abundance levels of autumn. In fact, David et al. (2007) reported that abundances of *A. tonsa* were significantly and negatively correlated with suspended particulate matter concentrations and positively and significantly correlated with water temperature and salinity. *Pseudocalanus elongatus* and p-*Calanus* showed higher abundance peaks in winter-spring, rising with higher values of salinity, whereas *Oncaea media* and *Temora stylifera* Dana, 1849 occurred at higher densities in summer along a salinity gradient.

In the Adriatic Sea system, inter-annual and seasonal variabilities in some copepod groups were respectively regarded as being due to over-flooding and warmer events (Kršinić et al. 2007). Nauplii were the most numerous fraction of all copepod groups, and showed the highest abundances during warmer and saltier conditions, which were referred to by the authors as an atypical distribution that may have been related to specific summer currents. Moreover, the highest abundances of harpacticoids were also reported with warmer and saltier conditions, with the highest values occurring in summer and autumn. The highest densities of calanoids and oithonids were observed in warmer months, which may have been related to the regulation of phytoplankton production levels during this period (Kršinić et al. 2007). Temperature is one of the most important factors controlling the biological cycle of copepods, by affecting juveniles and their growth rates (Hsiao et al. 2011). Moreover, while

adults may be tolerant to a wide range of salinities, earlier stages (nauplii and juveniles) might not be. Thus, the vulnerability of nauplii to environmental conditions, mainly salinity and temperature, can threaten the maintenance of a population, as they have narrow survival limits and suffer from high mortality (Chinnery and Williams 2004, Holste et al. 2009). Moreover, since copepods are an important component of the diet of benthopelagic invertebrates and peaks or absence of some copepod populations may influence the presence and life cycle of others populations (Carrasco et al. 2007), their dynamics allows us to infer information about ecological changes in aquatic systems (Hwang et al. 2010b). Therefore, more studies must be conducted on population distributions (and life-history stages) to understand relationships among species and their development as influenced by environmental factors. Such studies in combination with laboratory experiments will give us the chance to delineate the life cycle of species, further contributing to knowledge of populations and their contributions to trophic food webs.

REFERENCES

APHA. 1995. Standard methods for the examination of water and wastewater, 19th ed. Washington DC: American Public Health Association (APHA).

Ara K. 2001. Temporal variability and production of *Euterpina acutifrons* (Copepoda: Harpacticoida) in the Cananéia Lagoon estuarine system, São Paulo, Brazil. Hydrobiologia **453/454:** 177-187.

Azeiteiro UM, JC Marques, P Ré. 2000. Zooplankton assemblages in a shallow seasonally tidal estuary in temperate Atlantic Ocean (western Portugal: Mondego Estuary). Arq. Mus. Bocage **3:** 357-376.

Björnberg TKS. 1963. On the marine free-living copepods off Brazil. Bol. Inst. Oceanogr. Sao Paulo **13:** 3-142.

Carassou L, D Ponton. 2007. Spatio-temporal structure of pelagic larval and juvenile fish assemblages in coastal areas of New Caledonia, southwest Pacific. Mar. Biol. **150:** 697-711.

Carrasco N, I López-Flores, M Alcaraz, MD Furones, FCJ Berthe, I Arzul. 2007. First record of a *Marteilia* parasite

(Paramyxea) in zooplankton populations from a natural estuarine environment. Aquaculture **269:** 63-70.

Chinnery FE, JA Williams. 2004. The influence of temperature and salinity on *Acartia* (Copepoda: Calanoida) nauplii survival. Mar. Biol. **145:** 733-738.

Conover RJ. 1956. Oceanography and Long Island Sound 1952-1954. VI. Biology of *Acartia clausi* and *A. tonsa*. Bull. Bingham Oceanogr. Collect., pp. 156-233.

Cook KB, A Bunker, S Hay, AG Hirst, DC Speirs. 2007. Naupliar development times and survival of the copepods *Calanus helgolandicus* and *Calanus finmarchicus* in relation to food and temperature. J. Plankt. Res. **29:** 757-767.

Cunha PP, J Dinis. 2002. Sedimentary dynamics of the Mondego Estuary. *In* MA Pardal, JC Marques, MA Graça, eds. Aquatic ecology of the Mondego River basin. Global importance of local experience. Coimbra, Portugal: Imprensa da Universidade Coimbra, pp. 43-62.

David V, B Sautour, P Chardy. 2007. Successful colonization of the calanoid copepod *Acartia tonsa* in the oligo-mesohaline area of the Gironde estuary (SW France) - natural or anthropogenic forcing? Estuar. Coast. Shelf Sci. **71:** 429-442.

Dias CO, AV Araujo, R Paranhos, SLC Bonecker. 2010. Vertical copepod assemblages (0-2300 m) off southern Brazil. Zool. Stud. **49:** 230-242.

Dolédec S, D Chessel. 1994. Co-inertia analysis: an alternative method for studying species-environment relationships. Freshw. Biol. **31:** 277-294.

Gaudy R. 1972. Contribution à la connaissance du cycle biologique des copepods du Golfe de Marseille. Etude du cycle biologique de quelques espèces caractéristiques. Tethys **2:** 175-242.

Gonçalves AMM, M De Troch, SC Marques, MA Pardal, UM Azeiteiro. 2010a. Spatial and temporal distribution of harpacticoid copepods in Mondego Estuary. J. Mar. Biol. Assoc. UK **90:** 1279-1290.

Gonçalves AMM, MA Pardal, SC Marques, M De Troch, UM Azeiteiro. 2010b. Distribution and composition of small-size zooplankton fraction in a temperate shallow estuary (western Portugal). Fresenius Environ. Bull. **19:** 3160-3176.

Hafferssas A, R Seridji. 2010. Relationships between the hydrodynamics and changes in copepod structure on the Algerian coast. Zool. Stud. **49:** 353-366.

Hansen FC, C Möllmann, U Schütz, HH Hinrichsen. 2004. Spatio-temporal distribution of *Oithona similis* in the Bornholm Basin (central Baltic Sea). J. Plankt. Res. **26:** 659-668.

Hwang JS, R Kumar, HU Dahms, LC Tseng, QC Chen. 2010a. Interannual, seasonal, and diurnal variations in vertical and horizontal distribution patterns of 6 *Oithona* spp. (Copepoda: Cyclopoida) in the South China Sea. Zool. Stud. **49:** 220-229.

Hwang JS, R Kumar, CW Hsieh, AY Kuo, S Souissi, MH Hsu et al. 2010b. Patterns of zooplankton distribution along the marine, estuarine, and riverine portions of the Danshuei ecosystem in northern Taiwan. Zool. Stud. **49:** 335-352.

Holste L, MA Peck. 2006. The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): a laboratory investigation. Mar. Biol. **148:** 1061-1070.

Holste L, MA St John, MA Peck. 2009. The effects of temperature and salinity on reproductive success of *Temora longicornis* in the Baltic Sea: a copepod coping with a tough situation. Mar. Biol. **156:** 527-540.

Hsiao SH, TH Fang, CT Shih, JS Hwang. 2011. Effects of the Kuroshio Current on copepod assemblages in Taiwan. Zool. Stud. **50:** 475-490.

Incze LS, T Ainaire. 1994. Distribution and abundance of copepod nauplii and other small (40-300 μm) zooplankton during spring in Shelikof Strait, Alaska. Fish. Bull. **92:** 67-78.

Isari S, S Psarra, P Pitta, P Mara, MO Tomprou, A Ramfos et al. 2007. Differential patterns of mesozooplankters' distribution in relation to physical and biological variables of the northeastern Aegean Sea (eastern Mediterranean). Mar. Biol. **151:** 1035-1050.

Kâ S, JS Hwang. 2011. Mesozooplankton distribution and composition on the northeastern coast of Taiwan during autumn: effects of the Kuroshio Current and hydrothermal vents. Zool. Stud. **50:** 155-163.

Kršinić F, B Dubravka, R Precali, R Kraus. 2007. Quantitative variability of the copepod assemblages in the northern Adriatic Sea from 1993 to 1997. Estuar. Coast. Shelf Sci. **74:** 528-538.

Lam-Hoai T, D Guiral, C Rougier. 2006. Seasonal change of community structure and size spectra of zooplankton in the Kaw River estuary (French Guiana). Estuar. Coast. Shelf Sci. **68:** 47-61.

Limnologisk Metodik. 1992. Ferskvandsbiologisk Laboratorium. Københavns Universitet (ed.), Akademisk Forlag, København.

Marques SC, UM Azeiteiro, F Martinho, I Viegas, MA Pardal. 2009. Evaluation of estuarine mesozooplankton dynamics at a fine temporal scale: the role of seasonal, lunar, and diel cycles. J. Plankt. Res. **31:** 1249-1263.

Martinho F, R Leitão, I Viegas, M Dolbeth, JM Neto, HN Cabral, MA Pardal. 2007. The influence of an extreme drought event in the fish community of a southern Europe temperate estuary. Estuar. Coast. Shelf Sci. **75:** 537-546.

McAlice BJ. 1981. On the post-glacial history of *Acartia tonsa* (Copepoda: Calanoida) in the Gulf of Maine and the Gulf of St. Lawrence. Mar. Biol. **64:** 267-272.

Mendes S, MJ Fernández-Gómez, P Resende, MJ Pereira, MP Galindo-Villardón, UM Azeiteiro. 2009. Spatio-temporal structure of diatom assemblages in a temperate estuary. A STATICO analysis. Estuar. Coast. Shelf Sci. **84:** 637-644.

Morgado F, C Quintaneiro, E Rodrigues, MR Pastorinho, P Bacelar-Nicolau, L Vieira, UM Azeiteiro. 2007. Composition of the trophic structure of zooplankton in a shallow temperate estuary (Mondego Estuary, western Portugal). Zool. Stud. **46:** 57-68.

Mouny P, JC Dauvin. 2002. Environmental control of meso-zooplankton community structure in Seine estuary (English Channel). Oceanol. Acta **25:** 13-22.

Parsons TR, Y Maita, CM Lally, eds. 1985. A manual of chemical and biological methods for seawater analysis. Oxford, UK: Pergamon Press.

Portuguese Water Institute (Instituto da Água). 2008. Available at http://snirh.inag.pt Accessed Oct. 2010.

Portuguese Weather Institute (Instituto de Meteorologia). 2008. Available at http://web.meteo.pt Accessed Oct. 2010.

Primo AL, UM Azeiteiro, SC Marques, F Martinho, MA Pardal. 2009. Changes in zooplankton diversity and distribution pattern under varying precipitation regimes in a southern temperate estuary. Estuar. Coast. Shelf Sci. **82:** 341-347.

Richmond EC, DS Wethey, SA Woodin. 2007. Climate change and increased environmental variability: demographic responses in an estuarine harpacticoid copepod. Ecol. Model. **209:** 189-202.

Simier M, L Blanc, F Pellegrin, D Nandris. 1999. Approche simultanée de K couples de tableaux: apllication à l'étude des relations pathologie végétale-environnement. Rev. Stat. Appl. **47:** 31-46.

Simier M, C Laurent, JM Ecoutin, JJ Albaret. 2006. The Gambia River estuary: a reference point for estuarine fish assemblages studies in West Africa. Estuar. Coast. Shelf Sci. **69:** 615-628.

Strickland JDH, TR Parsons. 1972. A practical handbook of seawater analysis. 2nd ed. Fish. Res. Board Can. Bull. **167:** 1-311.

Tackx MLM, N Pauw, R van Mieghem, F Azémar, A Hannouti, S van Damme et al. 2004. Zooplankton in the Schelde estuary, Belgium and the Netherlands. Spatial and temporal patterns. J. Plankt. Res. **26:** 133-141.

Thioulouse J, D Chessel. 1987. Les analyses multitableaux en écologie factorielle. I. De la typologie d'état à la typologie de fonctionnement par l'analyse triadique. Acta Oecol.-Oec. Gen. **8:** 463-480.

Thioulouse J, D Chessel, S Dolédec, JM Olivier. 1997. ADE-4: a multivariate analysis and graphical display software. Stat. Comput. **7:** 75-83.

Thioulouse J, M Simier, D Chessel. 2004. Simultaneous analysis of a sequence of paired ecological tables. Ecology **85:** 272-283.

Uriarte I, F Villate. 2005. Differences in the abundance and distribution of copepods in two estuaries of the Basque coast (Bay of Biscay) in relation to pollution. J. Plankt. Res. **27:** 863-874.

Villate F, A Ruiz, J Franco. 1993. Summer zonation and development of zooplankton populations within a shallow mesotidal system: the estuary of Mundaka. Cah. Biol. Mar. **34:** 131-143.

Villate F, I Uriarte, X Irigoien, G Beaugrand, U Cotano. 2004. Oceanography and marine environment of the Basque country. *In* Á Borja, M Collins, eds. Zooplankton communities. Amsterdam: Elsevier Oceanography Series, pp. 395-423.