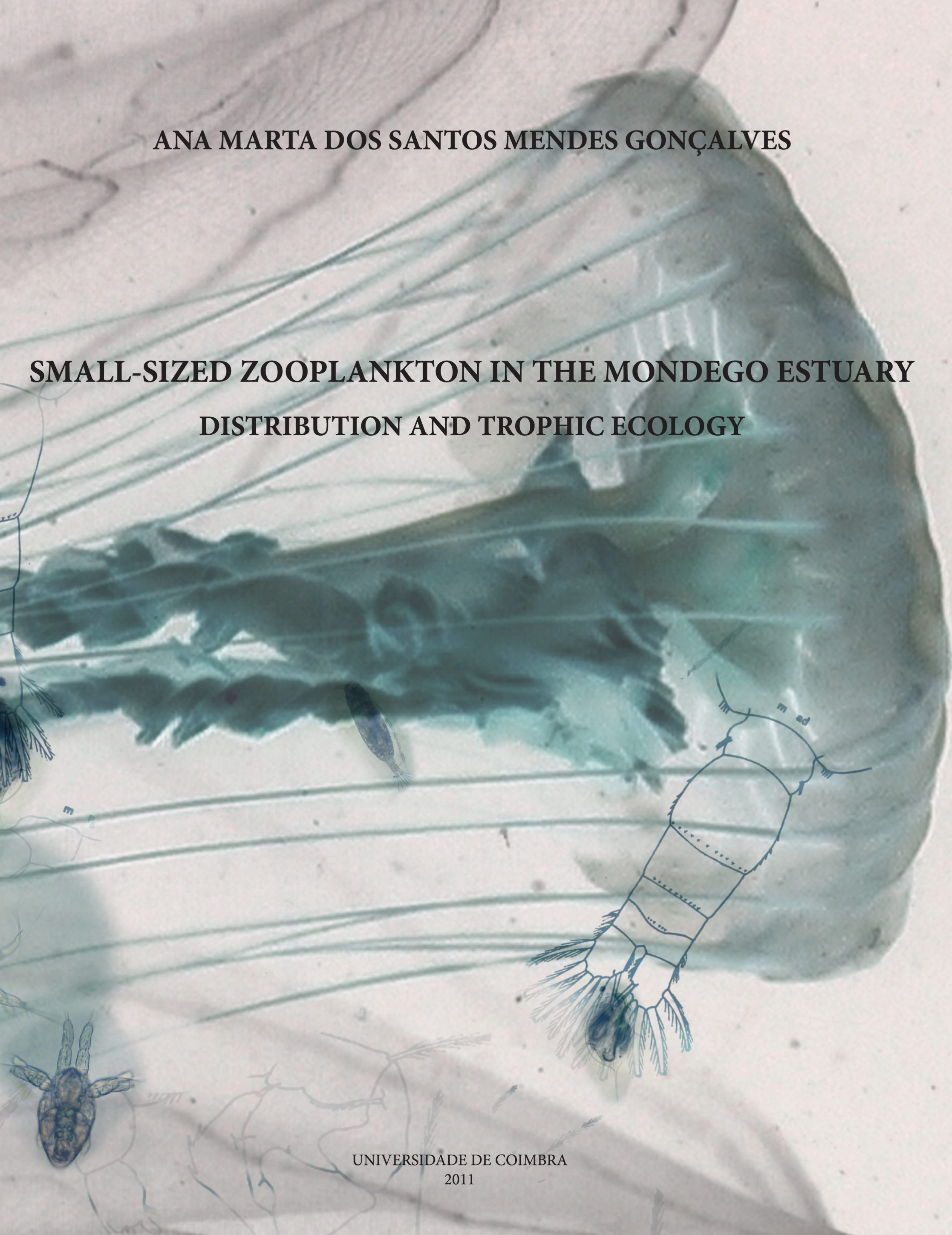

ANA MARTA DOS SANTOS MENDES GONÇALVES

SMALL-SIZED ZOOPLANKTON IN THE MONDEGO ESTUARY

DISTRIBUTION AND TROPHIC ECOLOGY

UNIVERSIDADE DE COIMBRA
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To Nuno Henrique, my beloved son

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Your love and smile makes everything worthwhile.

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GENERAL INTRODUCTION

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ABSTRACT

The main aims of this thesis were to study the spatio-temporal dynamics and distribution of small-sized zooplankton communities in the Mondego estuary (Portugal), based on field surveys conducted from 2005 to 2007, and to determine fatty acid composition and dietary preferences of zooplankton species in the estuarine system. In addition, the influence of physical conditions (e.g. diel, tidal and lunar cycles) and environmental variables (e.g. temperature and salinity) in the distribution of zooplankton communities were determined. The occurrence of a severe drought during the study period also allowed examining the effects of this extreme weather event on Copepoda ecology.

In chapter I it was studied the distributional spatio-temporal patterns of the small-sized zooplankton communities, collected with a 63 µm mesh sized net, and identified the major environmental parameters influencing the distribution of these assemblages. A total of 84 taxa were identified at both arms of the Mondego estuary. Copepods proved to be the main group of zooplankton community, with naupliar stages presenting the highest abundances (80.3%) followed by copepodites (14.4%). Marine species occurred at downstream areas and at the mouth of the estuarine system, whereas freshwater and estuarine species dominated in the middle and upper estuary. Indeed, a redundancy data analysis (RDA) showed a direct correlation between marine species and warmer and saline waters, whereas freshwater and estuarine species were positively correlated with colder waters, low salinity concentrations and high concentrations of nutrients. The abundances of copepods (adults) recorded in this study suggest an adaption of the earlier copepods stages to hydrological variations.

The Copepoda community was analyzed in more detail being possible to examine the response of copepod assemblages (nauplii, copepodites and adults) to climatic variability (extreme drought) and to seasonal, lunar, tidal and diel cycles. During sample collections were used a 63 µm and 335 µm mesh sized nets. The STATICO method was carried out to analyze the correlation between species distribution and forcing factors. Therefore, the effects of a severe drought on Copepoda ecology were assessed in chapter II. Monthly sampling were conducted during three-year period: 2005 (an extreme drought year), 2006 and 2007 (regular climatic years). Copepoda assemblages presented a clear seasonal pattern that superimposed to the inter-annual variability. Furthermore, the extreme drought recorded in 2005 was responsible for a higher dominance of marine species that remain

along the next regular climatic years (2006 and 2007). In chapter III were studied the short-term variations on the structure, composition and dynamics of the main copepod fraction of the estuarine system under the influence of physical forcing factors and environmental parameters. Spring-spring tide revealed to be the situation that best expressed the stable part of copepod-environment dynamics. This study also reported the main influence of depth and tidal currents in vertical migrations of copepods species. In addition, a novelty about behavioral aspects of *Oithona nana* and copepodites stages was observed, revealing this Oithonidae species with a clearly resident estuarine distribution.

Following a more focused approach of the Copepoda community, in chapter IV is provided a checklist of benthic harpacticoid community from the Mondego estuary. Temporal and spatial distribution and composition of harpacticoid copepods were assessed and were also identified the major hydrological parameters influencing their seasonal distribution. The north arm was characterized by higher densities of adults, whereas juveniles occurred mainly in the south arm of the estuary. Adults and copepodites of *Euterpina acutifrons* and *Paronychocamptus nanus* were the most dominant harpacticoid species, with the former species occurring predominantly at downstream areas, while *P. nanus* dominated the upper areas.

In chapter V was determined the fatty acid (FA) composition and dietary preferences of copepod and cladoceran species. A multivariate analysis revealed seasonal differences on FA composition of zooplanktonic organisms, reporting maximal concentrations and diversity of total FAs in winter and spring. In general, cladoceran species revealed a herbivore behavior, whereas copepod species revealed to be omnivorous, with the exception of *Calanipeda aquae dulcis* that showed a bacterivorous behavior. In addition, estuarine and freshwater species showed a higher and more diverse content of saturated and unsaturated fatty acids rather than marine species.

In this study it was determined the influence of environmental and physical forcing factors variation in zooplankton distribution (including their earlier life stages). It was also evaluated the contribution of zooplanktonic species in trophic food webs, in terms of FA composition. Indeed, zooplankton is a highly diverse group and the principal link in matter and energy transfer between primary producers and higher trophic levels making them extremely important in aquatic ecosystems food webs and thus, in ecological studies.

RESUMO

A presente tese teve como objectivos principais: 1) descrever a dinâmica espacio-temporal e a distribuição das comunidades de zooplâncton de pequenas dimensões ao longo do estuário do Mondego (Portugal), tendo como base campanhas de amostragem realizadas entre 2005 e 2007, e 2) determinar a composição de ácidos gordos e as preferências alimentares de espécies de zooplâncton no sistema estuarino. Foi ainda determinada a influência de parâmetros físicos (por exemplo: ciclos diários, lunares e de maré) e de parâmetros ambientais (por exemplo: temperatura e salinidade) na distribuição das comunidades de zooplâncton. A ocorrência de uma seca extrema durante o período de amostragem também permitiu analisar os efeitos de eventos climáticos extremos na ecologia dos copépodes.

No capítulo I estudaram-se os padrões de distribuição espacio-temporal das comunidades de zooplâncton, capturadas com malhagem de 63 µm, e identificaram-se os principais parâmetros ambientais que influenciam a distribuição dessas comunidades. No total foram identificados 84 *taxa* em ambos os braços do estuário do Mondego. Os copépodes foram o grupo dominante nas comunidades de zooplâncton, em que os nauplii apresentaram as maiores abundâncias (80,3%) seguidos dos estádios de juvenis (14,4%). As espécies marinhas apresentaram uma distribuição mais a jusante, preferindo águas mais salinas, enquanto as espécies de água doce e estuarinas surgiram principalmente no interior e a montante do estuário. Esta distribuição é explicada numa análise de Redundância (RDA) que mostra que as espécies marinhas estão directamente correlacionadas com águas mais quentes e salinas enquanto as espécies estuarinas e de água doce preferem águas mais frias, de baixa salinidade e com elevadas concentrações de nutrientes. Por outro lado, as abundâncias observadas de copépodes adultos sugerem uma adaptação às variações hidrológicas por parte dos primeiros estádios de copépodes.

Neste trabalho analisou-se mais pormenorizadamente a comunidade de copépodes (nauplii, juvenis e adultos), o que permitiu examinar as suas respostas às variações climáticas (seca extrema) e também aos ciclos sazonais, lunares, diários e de maré. As amostragens foram realizadas com redes de malhagem de 63 µm e 335 µm. O método STATICO foi usado para analisar a correlação entre a distribuição das espécies e os factores físicos e ambientais. Deste modo, no capítulo II foi avaliado o efeito de uma seca extrema na ecologia dos copépodes. As amostragens mensais foram realizadas ao longo de 3 anos: 2005

(definido como um ano de seca extrema), 2006 e 2007 (definidos como anos regulares em termos climáticos). As comunidades de copépodes apresentaram um padrão claramente sazonal que se sobrepôs à variabilidade interanual. Por outro lado, os meses bastante secos de 2005 conduziram a um domínio das espécies marinhas no estuário, padrão este que se manteve nos anos seguintes. No capítulo III estudaram-se as alterações de curta escala na estrutura, composição e dinâmica das principais espécies de copépodes do sistema estuarino, sob a influência de factores físicos e de parâmetros ambientais. As marés vivas na Primavera revelaram ser a situação que melhor explica a estabilidade na dinâmica entre copépodes e o ambiente. Neste estudo foi também referida a principal influência da profundidade e das correntes de maré nas migrações verticais dos copépodes. O presente estudo revelou o comportamento residente e estuarino da espécie *Oithona nana* (e de juvenis), o qual até à data era desconhecido para o estuário do Mondego.

Seguindo um aprofundamento no conhecimento da comunidade de copépodes, no capítulo IV apresenta-se uma lista da comunidade de harpacticóides do estuário do Mondego. Neste estudo avaliou-se a distribuição espacio-temporal e a composição da comunidade de harpacticóides. Foram ainda identificados os principais parâmetros ambientais que influenciam a distribuição sazonal dos copépodes. Os resultados obtidos mostram que no braço norte existem maiores densidades de adultos, enquanto no braço sul predominam os estádios juvenis. As espécies de harpacticóides mais abundantes no estuário do Mondego são *Euterpina acutifrons* e *Paronychocamptus nanus. E. acutifrons* distribui-se principalmente a jusante enquanto *P. nanus* apresenta maiores ocorrências a montante.

No capítulo V determinou-se a composição de ácidos gordos e preferências alimentares de espécies de copépodes e de cladóceros. A análise multivariada identificou diferenças sazonais na composição de ácidos gordos nas espécies zooplanctónicas. Maior diversidade e maiores concentrações de ácidos gordos foram observadas nas espécies amostradas no Inverno e na Primavera. Em termos tróficos, e de um modo geral, as espécies de cladóceros mostraram um comportamento herbívoro, enquanto a omnívoria foi patente nas espécies de copépodes, com excepção da espécie *Calanipeda aquae dulcis* que se alimentou basicamente de bactérias. Por outro lado, as espécies estuarinas e de água doce apresentaram uma maior riqueza em ácidos gordos saturados e insaturados do que as espécies marinhas.

Neste estudo determinou-se a influência das variações ambientais e dos factores físicos na distribuição do zooplâncton (incluindo os primeiros estádios de vida). Foi também avaliada a contribuição das espécies de zooplâncton nas cadeias tróficas, reportando para a sua composição em ácidos gordos. Deste modo, a relevância e o destaque que o zooplâncton assume nas cadeias tróficas dos sistemas aquáticos e, por isso, em estudos ecológicos, estão estreitamente relacionados com a diversidade deste grupo e com a principal função que desempenha na tranferência de matéria e energia entre os produtores e os níveis tróficos superiores.

GENERAL INTRODUCTION

Zooplankton – their role in the estuarine ecosystems

Estuaries are amongst the most productive natural systems in the world, supporting a great variety of marine resources with economic potential (e.g. crustaceans, molluscs and fish). These ecosystems are transition zones between rivers and the sea and differ from both in terms of biotic (e.g. predation, competition) and abiotic conditions (e.g. temperature, salinity, food quantity and quality), presenting higher fluctuations than in marine or freshwater systems (David et al., 2005; Isari et al., 2007). Plankton (and mainly zooplankton) is known to be particularly sensitive to these variations because it is strongly influenced by climatic features and changes in hydrological conditions, resulting in communities that are continuously changing and adapting to environmental factors (Ara, 2001; Beaugrand et al., 2000). This also suggests that plankton is well adapted to changes in the available resources.

In the last decades it has been highlighted the importance of plankton (and mainly zooplankton) in the dynamic of aquatic food webs. Zooplankton plays a pivotal ecological role in terms of biomass and energy fluxes transference between primary producers and higher trophic levels, highlighting the importance of this group in ecological studies (Mclusky and Elliot, 2004). Furthermore, spatio-temporal scales of zooplankton communities' structure and composition are an important focus in ecological research (Melo Júnior et al., 2007). However, the dynamics and structure of the marine and estuarine zooplankton communities require the use of sampling methods that provide representative capture of the populations. There is extensive information available about zooplankton communities (Edwards and Richardson, 2004; Kirby et al., 2007; Lam-Hoai et al., 2006), some of them associated to the effects of eutrophication process (Anneville et al., 2007; Hsieh et al., 2011), extreme climatic events, such as severe droughts and floods (Hampton et al., 2008; Isari et al., 2007; Primo et al., 2009; Richmond et al., 2007), and the impact of fisheries (Reid et al., 2000). Nonetheless, data on the structure of smaller size fractions of zooplankton community are still rather scarce due to the difficulties in identification and to current use of nets with mesh sieves bigger than 200 µm, leading to underestimations of zooplankton communities as a whole (Calbet et al., 2001; Pitois et al., 2009; Sautour and Castel, 1998; Turner, 2004).

Ecological importance of small-sized zooplankton

In most mesozooplankton studies (Jiang-Shiou et al., 2007; Lam-Hoai et al., 2006; Marques et al., 2009; Primo et al., 2009) the abundance and biomass of zooplankton groups (including their developmental stages) have been either underestimated or ignored (Antacli et al., 2010; Gallienne and Robins, 2001; Tseng et al., 2011; Turner, 2004) due to the traditional mesh size employed (≥200 µm). Recent studies have shown that when appropriate net meshes of 100 µm or smaller are used, the abundance and sometimes the biomass of small zooplankton groups can vastly exceed those of larger ones. Gallienne and Robins (2001) reported the 200 µm mesh nets capture <10% of the mesozooplankton community in terms of numbers, while the biomass is underestimated by one-third and the secondary production by two-thirds. Moreover, the 200 µm mesh nets have been shown to capture one order of magnitude less than the 63 µm nets (Gallienne and Robins, 2001). In addition, Hwang et al. (2007) estimated losses of 70% to 92% of oithonid copepod individuals with a mesh size plankton net of 333 µm, whilst on other studies (e.g. Gallienne and Robins, 2001; Krsinic and Lucic, 1994) a retention of 3.20% to 21.30% of copepods have been shown through a plankton net of 280 µm mesh size. Riccardi (2010) estimated a loss of total abundance of 73% to 95% of smaller sized zooplankton fraction when compared the total abundance between 80 µm and 200 µm fractions. Moreover, the author reported copepod nauplii as the most abundant component on the zooplankton assemblages, followed by species of the genus *Oithona*. Indeed, the small cyclopoids of the genus *Oithona* have been shown often dominant in several aquatic ecosystems with a worldwide distribution (e.g. estuaries, open waters, tropical and sub-tropical waters), making *Oithona* one of the most important copepod in food webs (Gallienne and Robins, 2001; Porri et al., 2007; Turner, 2004). Similarly, Calbet et al. (2001) also stated significant differences between 200 µm and 53 µm mesh sized nets, reporting the abundance of total zooplankton >200 µm was underestimated by a factor of 4.4 using a 200 µm mesh net. The author found the abundance of the genera *Euterpina* and *Oithona* obtained with the 200 µm net was 24 times lower than the ones obtained with the 53 µm net, while for cladocerans and molluscs it was two times lower. Indeed, Calbet et al. (2001) reported copepods as the dominant zooplankton group, with copepod nauplii constituting the bulk of the community (representing 59% of the total zooplankton abundance). The second most abundant zooplankton group was cladocerans, followed by appendicularians, cirriped, mollusc and polychaeta larvae. This inadequate estimation of smaller zooplankton fraction is not only important in terms of species diversity and abundance but also for zooplankton-mediated fluxes, rates, ecological processes and for the modeling ecosystem dynamics, with consequent impacts on food-web and biogeochemical models (Riccardi, 2010; Tuner, 2004).

In the light of relatively-recent studies using fine plankton nets or comparing different mesh sized nets (Antacli et al., 2010; Jaspers et al., 2009; Jiang-Shiou et al., 2007; Riccardi, 2010; Tseng et al., 2011), adults and copepodites stages of cyclopoid genera (e.g. *Oncaea, Oithona* and *Corycaeus*), calanoid genera (e.g. *Acartia*, *Clausocalanus*, *Paracalanus*, *Pseudocalanus* and *Temora*), planktonic harpacticoids (e.g. *Euterpina* and *Microsetella*), naupliar stages, as well as gastropod, polychaeta and appendicularians larval stages have been historically underestimated or lost by larger mesh nets. A substantial proportion of the copepod assemblages are composed of small-sized species, which normally dominate the mesozooplankton. Small planktonic marine copepods (<1 mm in length) are undoubtedly the most abundant metazoans on Earth (Banse, 1995). Several authors (e.g. Calbet et al., 2001; Krsinic and Lucic, 1994; Nichols and Thompson, 1991; Saville, 1958) determined losses of smaller copepod taxa up to 92% for *Oithona*, 78% for *Microcalanus* and 52% for *Pseudocalanus* using a 250 μm mesh net. This small-sized fraction of copepods represent an important link in the food webs, being dominant grazers of phytoplankton, function as prey for higher trophic groups (e.g. fish larvae and other planktivores) and are central in the cycling of organic matter (Damotharan et al., 2010; Turner, 2004). Therefore, it is important to include the state of these communities in any investigation, being highlighted the importance of planktonic copepods in the dynamic of aquatic food webs and raised the importance of this group in ecological studies (Damathoran et al., 2010; Kršinić et al., 2007; McLusky and Elliot, 2004). Accordingly, it is of crucial importance to estimate the abundance and understand the spatio-temporal variations of small planktonic copepods, their trophic ecology and their biogenic fluxes. Furthermore, these communities are strongly influenced by environmental stressors, such as tidal currents (ebb and flood) and river flow, in estuaries. These advective processes are mainly responsible for biological mechanisms such as vertical migrations. Indeed, vertical migration behavior of the organisms aggregates them at certain depths, with the degree of migration closely related with tide currents, resulting at horizontal displacement or retention of copepod species (including earlier life stages).

Diel vertical migration and selective horizontal transport of copepod communities: dynamics structure responses (fluxes)

In the last decades diel vertical migration (DVM) accomplished by horizontal transport in invertebrates has been recognized as a key feature. Nevertheless only a few years ago it has been reported over tidal time scales (Forward Jr. and Tankersley, 2001). In coastal and estuarine areas tidal currents oscillate in a pattern of flood- and ebb-tide currents, being the selective tidal-stream transport (STST) characterized as flood- or ebb-tide transport according to the phase of the tide is used for transport (Forward Jr. and Tankersley, 2001). Thus, some organisms use ebb-tide transport for movement out of an estuary or offshore, whereas flood-tide transport is used for movement in the opposite direction. The transport by tidal currents is an efficient method for fast horizontal movements, mainly on earlier stages due to their limited swimming abilities. Even so, it also reduces the energy necessary to those movements in organisms reported as good swimmers (Metcalfe et al., 1990). Moreover, STST is used mainly during the night when predation by visual predators is greatly reduced, and thus losses of individuals (Zeng and Naylor, 1997). A similar mechanism is associated with the larval releasing activity, where the spawning migration involves both flood- and ebb- transport of adults' females. Most of the studies on STST of invertebrates have focused crustaceans, mainly crabs and other decapods. In the life cycle of these groups, ovigerous females aggregate near the entrance to estuaries and, shortly before release their larvae, they migrate to the surface during ebb-tides, through the crepuscular phase which is probably an adaptation process to avoid predation by visual feeders (Paula, 1989; Zeng and Naylor, 1997). Females are transported passively seaward where they release their larvae in coastal areas, being transported back into the estuary on flood tides on subsequent nights (Tankersley et al., 1998). Indeed, several studies focused the synchronism between the rhythmic cycles of larval export and the light and lunar phases, being larvae most often released around the time of spring tides at the new and full moon (Gonçalves et al., 2003; Paula, 1989; Queiroga et al., 1994, 1996, 1997). Another less studied factor is the migratory behavior of the different life history stages, only addressed on few species, mainly fish (e.g. the Atlantic salmon *Salmo salar* and the European ell *Anguilla anguilla*) and crabs (e.g. *Carcinus maenas* and *Callinectes sapidus*) (Forward Jr. and Tankersley, 2001). Indeed, spawning migration up-estuary or seaward involves undoubtedly ebb and flood tides transport. Notwithstanding the transport during the day or night varies among species, being consistent among life history stages within a species (Forward Jr. and Tankersley, 2001). On the other hand, the same authors reported that the exposure of zooplankton (and thus copepods) to environmental factors that show a gradient with depth, induce vertical movements in response to that change. For instance, during flood tide, with the organism descend to the bottom of the estuary (increasing depth scenario), salinity and hydrostatic pressure increase, whereas temperature decreases. Thus, the predicted behavioral response is an ascent. However, during ebb-tide, an organism in the bottom is exposed to a decrease in salinity and in hydrostatic pressure and an increase of temperature. Since all these changes evoke a descent in the water column, movement off the bottom is inhibited during ebb tide. A third situation is related to upon descending scenario where the organism experiences an increase of temperature and a decrease of salinity and hydrostatic pressure, while it is ascending from the bottom, that induce a downward movement. Still, once in the water column the animal experience very slow changes in salinity and temperature. However, other environmental factors, such as water turbulence in flood tide or slack water at the end of flood tide, may be detected by the organism predicting a behavioral response (Welch et al., 1999). Accordingly, it is now assumed that the vertical distribution patterns of copepods along the year are a dynamic complex structured response to tidal, diel and lunar cycles and to a gradient of environmental variables (e.g. temperature, salinity, hydrostatic pressure), as well as to reproductive cycles of predators (e.g. fish) and prey (e.g. phytoplankton) (Forward Jr. and Tankersley, 2001; Kingsford et al., 2002).

Harpacticoida Copepoda: ecological status and community characterization

Harpacticoids comprise one of the main components of marine meiofauna, constituting a diverse group and the second most abundant in what respect to meiofauna taxa (Huys and Boxshall, 1991; Suárez-Morales et al., 2006). These invertebrates are well adapted to different environments exploiting a wide range of habitats over the entire planet due to their taxonomic and ecological diversity (Chertoprud et al., 2010). A wide range of harpacticoid body forms has accompanied the exploitation of these diverse habitats. Epibenthic or phytal species display a host of body shapes including lateral and dorsal-ventral compression of the body, extremely rounded or broadened cephalosomes and larger overall size, whereas most sediment harpacticoids are vermiform or torpedo shaped (Noodt, 1971). Many harpacticoids (particularly phytal and epibenthic species) are active dispersers and occasionally enter the water column by themselves (Boeckner et al., 2009; Chertoprud et al., 2010). Once in the water column, organisms can be transported to long distances. This behavior together with resuspension mechanisms turns out this fraction to be an important component in the zooplankton collections (Leandro et al., 2007; Tseng et al., 2011; Wen-Tseng et al., 2004).

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.

Despite the impressive progress made in their taxonomy (Boxshall and Halsey, 2004; Wells, 2007) and ecology (Giere, 2009; Mokievsky, 2009), a comprehensive analysis of harpacticoid biogeography has not been carried out in the last 25 years. The data about the species composition of harpacticoids from different regions of the world are few and disembodied (Veit-Köhler et al., 2010). On the other hand, most of the publications available present only restricted information about the group (e.g. descriptions of species of individual biotopes, such as descriptive keys or catalogs, or revisions of selected families or genera), not covering a series of species from earlier studies (Chertoprud et al., 2010). For instance, studies concerning composition of marine harpacticoid fauna (Huys et al., 1996; Lang, 1948, 1965) are restricted to a limited number of habitats or specific areas such as the Gulf of Mexico and other tropical areas (Fiers, 1995, 1996; Fleeger and Clark, 1979; Rutledge and Fleeger, 1993; Suárez-Morales et al., 2000, 2006). In Portugal, few works have been carried out focusing on harpacticoid copepods' ecology and taxonomy (Morgado, 1997) and most of them are focused on a single species (Galhano, 1968; Noodt and Galhano, 1969). Indeed, general knowledge on harpactiocid species' composition and distribution is still scarce and poorly understood (Chertoprud et al., 2010; Seifried, 2004). Even more, in the best studied regions regarding meiobenthos communities, information about harpactioid diversity and species composition remains remarkably limited (Geerardyn et al., 2009).

Importance of fatty acid profiles in trophic food webs

Fatty acids (FAs) constitute the main part of the lipids in aquatic organisms, being one of the most important molecules transferred across the plant-animal interface in aquatic food webs (Pasquaud et al., 2007). FAs have an enormous influence on the integrity and physiology of living organisms and are involved in many vital functions of aquatic individuals (Dalsgaard et al., 2003). Moreover, the so-called essential FAs are produced by bacteria, plants and algae and cannot be synthesized *de novo* by primary consumers, constituting useful trophic markers because they are assimilated and retained by animals (Dalsgaard et al., 2003). Among them, are the highly unsaturated fatty acids (HUFA) of n-3 fatty acids (e.g. eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) that play a key role in the health and function of all animals at all trophic levels, including plankton invertebrates, fish and humans. These HUFAs cannot be synthesized *de novo*, or at least not in sufficient amount for somatic growth, reproduction and survival by consumers (Arts et al., 2009; Wacker and Von Elert, 2001). Furthermore, lipids components are very sensitive to stressors and environmental changes (Arts et al., 2009). Thus, large-scale processes such as eutrophication and global warming may act either independently, or together, leading to an overall decrease in HUFA production in aquatic ecosystems with possible negative implications for the surrounding terrestrial communities. In the last decades the interest on fatty acid composition of aquatic organisms has been increasing. The structure of these compounds is easily recognizable and quite well detected in primary producers (Pasquaud et al., 2007). Feeding behaviour is the basic process in the food web for the transfer of energy and material from lower to higher trophic level. Therefore, biochemical techniques allow the identification of trophic relationships between primary producers-primary consumers. Marine zooplankton, mainly marine copepod species, is a well-documented group due to their use as live feed for commercial species, mainly marine fish (Arts et al., 2009; Dalsgaard et al., 2003; Perumal et al., 2010). Still, the feeding ecology of small copepods is less well-known in comparison of adults of larger copepod species. Copepods are the dominant group of mesozooplankton, followed by cladocerans, playing a key role in the food web as they form a link between primary producers and secondary consumers (Guschina and Harwood, 2009; Richmond et al., 2007). There are important differences between both zooplankton guilds, especially regarding their impact on the lower trophic levels, either directly via feeding or indirectly by influencing nutrient cycling (De Mott, 1995; Hessen and Lyche, 1991). Therefore, determination of biochemical composition of copepod and cladoceran communities has become important to understand their physiological functions, metabolism and nutritive value, as this is very relevant for the energy transfer in aquatic ecosystems and secondary production.

The study system: Mondego estuary

In the last years several differences in the climate of Portugal have been recorded when compared to the general climate patterns for the last century (INAG – Portuguese Water Institute, http://snirh.inag.pt; IM – Portuguese Weather Institute, http://web.meteo.pt) (Fig. 1). In 2005 an extreme drought was recorded with precipitation and freshwater discharge values much lower than the 1971-2000 average (2005: 486.1 mm against the mean annual of 1030.6mm), causing one of the largest droughts of the 20th century in Portugal. A severe reduction was evidenced in freshwater flow with the lowest value in 2005 almost 48-fold lower the highest in 2006. Mondego estuary suffered the influence of this climatic events that forced biological and ecological responses from aquatic communities. The field work of the present thesis addresses to this estuarine system.

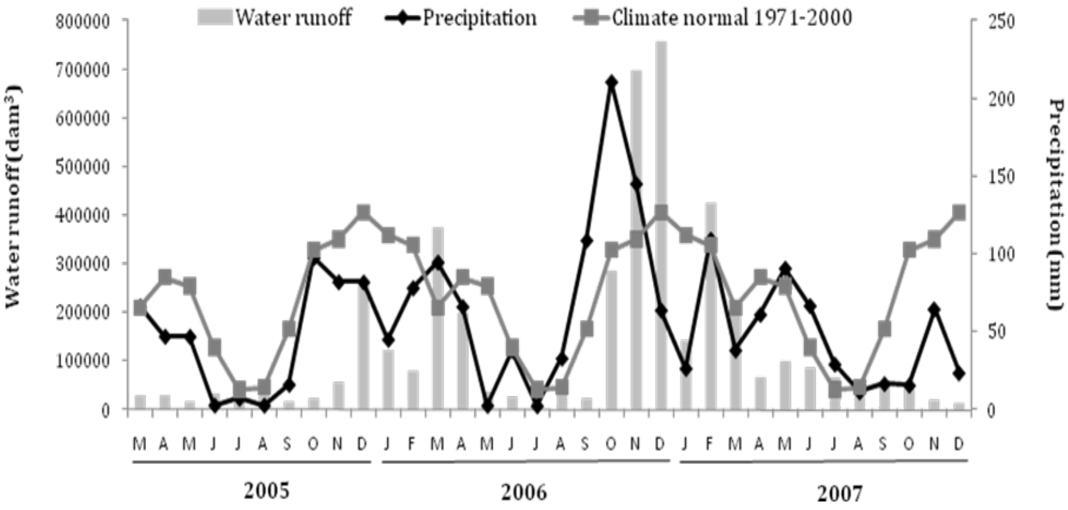

Figure 1 – Monthly precipitation (mm) in Mondego estuary from 2005 to 2007. Grey filled squares represent monthly average of 1971 to 2000 (http://snirh.inag.pt)

Mondego estuary is located in the Atlantic coast of Portugal, near to Figueira da Foz city (40º08' N, 8º50'W), a warm temperate region, with a Mediterranean temperate climate. The Mondego estuary is a small mesotidal system with a area of 8.6 km^2, divided in two arms, north and south, separated about 7 km from the shore joining again near the mouth by an alluvium-formed island - the Murraceira island (Fig. 2). The two arms present different hydrological characteristics. The north arm is deeper (4-8m during high tide, tidal range about 1 to 3 m), presents a low residence time (<1day) and is the location of the main navigation channel, serving the Figueira da Foz commercial harbour. The south arm is shallower (2-4m deep during high tide, tidal range about 1 to 3m), has higher residence times (2-8 days) and was largely silted up, especially in the upstream areas, until 2006, forcing most of the freshwater discharge to flow throughout the northern arm. The water circulation in the southern arm is predominantly due to the tidal cycle and the freshwater input of a small tributary, the Pranto River, which is artificially controlled by a sluice, according to the water needs of the Mondego valley on rice fields. The Mondego estuary suffers from direct and indirect human effects related to high inputs of nutrients from agricultural fields and aquacultures, pollutants input from industrial and domestic discharges, harbour facilities and economical growth at the regional scale. Since the late 80's, the Mondego estuary showed symptoms of eutrophication as a result of anthropogenic activities. Several interventions were performed in the lower Mondego valley in order to improve irrigation efficiency of the agricultural fields, which included the construction of channels, regularization of margins to reduce floods and the construction of dams to regulate the water level in the fields (Cardoso et al., 2007). Since 1998 the system is gradually recovering after the implementation of mitigation measures (freshwater circulation enhancement, reduction of nutrient loading and seagrass bed protection) which enhanced environmental quality (Cardoso et al., 2005; Dolbeth et al., 2007). In 2006 was performed another physical intervention in the estuary in order to re-establish the connection of the south arm riverhead, enlarging the upstream communication between both arms, improving a higher water circulation and thus reducing the water residence time in the south arm.

In the last two decades several works were conducted in the Mondego estuary providing a large database and a further knowledge of the system on several areas such as: nutrient dynamics (e.g. Lillebø et al., 2007), intertidal and subtidal benthic invertebrates (e.g. Cardoso et al., 2007; Viegas et al., 2007), plankton communities (e.g. Azeiteiro et al., 1999; Marques et al., 2007; Primo et al., 2009); fish community (e.g. Dolbeth et al., 2008; Martinho et al., 2008), secondary production (e.g. Dolbeth et al., 2007) and bird species (e.g. Lopes et al., 2006).

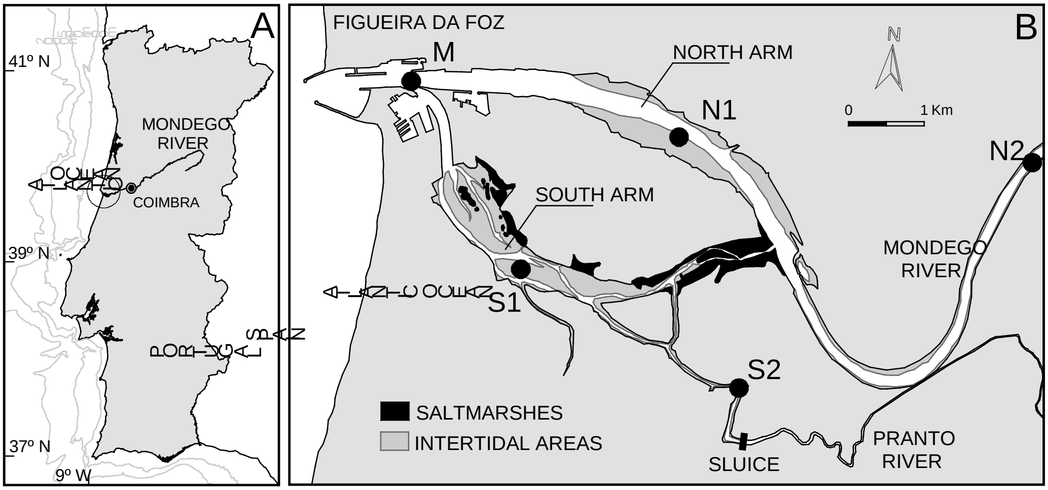

Figure 2 – Location of the Mondego estuary on the western coast of Portugal and the five sampling stations within the estuary: (M – mouth station, N1 and N2 – northern arm stations, S1 and S2 – southern arm stations).

Considering the plankton research, Gonçalves (1991) studied the spatio-temporal distribution of the zooplankton community fractions >200 µm and >500 µm in the Mondego estuary without addressing copepods assemblages, whereas Azeiteiro (1999) considered in his study the zooplankton fractions >200 µm and >335 µm of the south arm. More recently, Marques (2009) conducted her work on the dynamics and composition of mesozooplankton assemblages at both arms of the estuarine system, using a 335 µm a mesh sized net. Her study also approached the effects of the response of zooplankton community to climatic variability as well as to tidal and diel cycles. However, the small-sized fraction of zooplankton community of the Mondego estuary has not been studied, which limited the knowledge of this community as a whole.

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GENERAL AIMS AND THESIS OUTLINE

In the Mondego estuary earlier studies (Azeiteiro et al., 1999; Marques et al., 2007, 2008; Primo et al., 2009 and references therein) focused only macroplanktonic taxa being the ecosystem widely well-documented in terms of mesozooplankton (>200 or >335 µm) communities. However there is a single work performed by Vieira et al. (2003) that used a fine mesh net (63 µm), but only the southern arm was studied, contributing to a limited overview of the system. Therefore, studies on the small sized fraction of zooplankton (>63 µm) and on early development stages of crustaceans comparing the communities of both arms have not been carried out. Additionally, zooplankton plays a key role in the food web as they form a link between primary producers and secondary consumers. Despite their importance in the estuarine food chains, works concerning biochemical composition of zooplankton species in the Mondego estuary in order to determine dietary preferences and their contribution in energy transfer in the food web is lacking so far.

Therefore, this thesis aims to address 1) the influence of forcing factors in the spatio-temporal dynamics of small-sized fraction and earlier development stages of zooplankton assemblages and 2) the characterization of zooplankton species-specific fatty acids profiles and dietary preferences in the Mondego estuary. Within the first goal were considered three specific objectives: 1.1) to determine the response of copepods assemblages to an extreme weather event (drought), 1.2) to understand the correlation between the distribution of copepod assemblages (naupliar, copepodites and adults) and physical properties of water column over seasonal, tidal, lunar and nycthemeral dynamics and 1.3) to study the composition and distribution of the harpacticoid community of the Mondego estuary over spatial and temporal (seasonal) environmental variations. Therefore, the thesis core is structured in five chapters, comprising five papers. The main aims of the present study are addressed at the five chapters that are summarized below. At the end of the thesis, a general discussion with main conclusions is presented, providing an overview and integration of the five chapters.

Chapter I

The first chapter investigates the spatio-temporal patterns of small-sized zooplankton fraction and earlier developmental stages, mainly of copepods, and compares the distribution and structure of the communities in both arms of the Mondego estuary.

Moreover, aims to identify the major environmental factors that may affect the distribution of these assemblages. "Distribution and composition of small-size zooplankton fraction in a temperate shallow estuary (Western Portugal)" represents the first study of small sized zooplankton communities in the Mondego estuary and its relationships to physical and chemical environmental forcing factors.

Chapter II

Global changes in weather patterns driven by extreme weather events (floods and/or droughts) may have severe repercussion in reproduction, migration and abundance of copepods and thus in multiple species of aquatic food webs. Hence, the second chapter deals with the response of the Mondego estuary Copepoda assemblages and their life history stages to a severe drought that occurred during the study period. One of the main objectives of "Response to climatic variability of Copepoda life history stages in a southern European temperate estuary" is to study the life-history stages (nauplii, juveniles and adults) of copepods under two contrasting environmental conditions (an extreme dry year and regular years). The other main goals of this chapter are: 1) to determine the major environmental parameters that explains copepods' dynamics, and 2) to determine inter-annual variations of copepods assemblages during an extreme drought. The partial triadic analysis (STATICO method) was used as it is particularly suited to study the relationship between zooplankton species' distribution and environmental factors when both vary over time. This study contributes to a further knowledge about inter-annual variations in abundance and diversity of copepod community, assessing a holistic and integrative view of an ecosystem to global climate change.

Chapter III

Since several decades diel vertical migration accomplished by horizontal transport in invertebrates (and thus in copepod communities) has been recognized. In estuarine and coastal areas the transport by tidal currents is an efficient method for fast horizontal movements, mainly to earlier stages. Thus, in chapter III is examined seasonal, tidal, lunar and diel vertical migrations accomplished by horizontal movements on the main copepod fraction of the Mondego estuary. "Seasonal and diel vertical behavior of Copepoda assemblages (naupliar, copepodites and adults) at the boundary of estuarine and coastal waters" aims to analyze the distribution of the main copepod community and life history stages of the Mondego estuary, associating them with physical factors (e.g. lunar phase, diel cycles, depth, tidal currents). Moreover, other objective of this chapter is to identify the factors that contribute to vertical migratory behavior promoting retention or displacement in the estuarine system. Additionally, the distribution of copepod species was compared between the inner stations (north and south arms) and the mouth of the Mondego estuary in order to encompass species' life cycles distribution. Indeed, a first characterization of life cycles of the main copepod species was attempted in order to relate the distribution of the different life history stages of each species.

Chapter IV

Harpacticoids are an important food source for higher trophic levels, whose reduction may cause profound impacts in many other estuarine species. Despite harpacticoids represent a less abundant fraction in copepod community, this order constitutes a diverse group and the second most abundant in what respect to meiofauna taxa. In chapter IV the first characterization of the composition and distribution of the Harpacticoida Copepoda fauna in the Mondego estuary is studied. Indeed, the first checklist of the harpacticoid copepods is provided in this chapter. Objectives of "Spatial and temporal distribution of harpacticoid copepods in Mondego estuary" are to analyse the temporal and spatial patterns of Harpacticoida community (copepodites and adults) at the two contrasting arms and to identify the major environmental factors that influence their seasonal distribution.

Chapter V

Copepods are the dominant group of mesozooplankton, followed by cladocerans. Both groups play a crucial role in the food web as they form a link between primary producers and secondary consumers. Thus, it is of main importance to determine their nutritive value in aquatic ecosystems. In Chapter V the characterization of fatty acid profiles of zooplankton species is determined. "Species-specific fatty acids profiling reveals functional shifts in zooplankton in a temperate estuary" aims to examine the fatty acids composition of copepods and cladocerans species in order to trace spatio-temporal (seasonal) changes in dietary preferences and in fatty acids profiles of zooplankton species.

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CHAPTER I

Distribution and composition of small-size zooplankton fraction in a temperate shallow estuary (Western Portugal)

Chapter I is published in the form of a manuscript in Fresenius Environmental Bulletin – 19(12b): 3160-3176.

ABSTRACT

The variation of small zooplankton abundance and distribution patterns was studied in a shallow temperate southern European estuary (Mondego estuary, Portugal). Monthly plankton sampling was carried out from February 2005 to July 2006 at five stations, in both arms of the estuary – north and south, with subsurface 63 μm mesh tows. Concomitant hydrological parameters were measured. 84 zooplankton taxa were identified in the northern and southern arms stations. Copepods were the most abundant group (95.6% of the total abundances). Nauplii were the most abundant copepod development stage (80.3% of the total), followed by copepodites (14.4%). Marine zooplankters were reported at the mouth of the estuary and downstream stations while freshwater and estuarine species dominated in the inner part of the estuary. Statistical analysis (RDA) correlated marine species with high salinity and temperature while freshwater and estuarine species were correlated with low salinity and temperature and high nutrient concentrations. The relationship between species distribution and environmental parameters suggests adaptive strategies of the first stages of copepods to hydrological variations which explain the adults' abundances.

KEYWORDS

Zooplankton, Distribution, Abundance, RDA analysis, Environmental parameters, Temperate shallow estuary.

INTRODUCTION

High biological productivity (Cardoso et al., 2008; Paerl, 2006) and the provision of ecological and ecosystem services (Kennish, 2002; Paerl, 2006), makes coastal ecosystems one of the most important environments on Earth. The Mondego estuary is a temperate shallow ecosystem located along the western coast of Portugal. It is subjected to a variety of anthropogenic stressors (e.g. eutrophication) leading to biological impoverishment (Dolbeth et al., 2007; Martinho et al., 2008). This ecosystem is widely well-documented in terms of benthic and mesozooplankton (> 200 and 335 μm) communities (Azeiteiro et al., 2000; Dolbeth et al., 2008; Gonçalves et al., 2003; Primo et al., 2009 and references therein). These earlier studies (with > 200 or > 335 μm) sampled only adult and large copepodites of the dominant organisms in the estuary: copepods and other macroplanktonic taxa. However studies on the small size fraction of zooplankton (> 63 µm), and on early development stages of crustaceans with the aim to compare the ecology of different life stages (e.g. adults, juveniles – copepodites, and nauplii) have not been carried out. Furthermore, information about smaller zooplankton is lacking in literature (Chinnery and Williams, 2004; Porri et al., 2007), especially on estuarine areas of south Europe. Still, there was a single work performed by Vieira et al. (2003) that used a fine mesh net (63 µm), but only the southern arm was studied, contributing to a limited overview of the system.

Earlier life-history stages (mainly nauplii stages) are pivotal for the further development and growth rate of juveniles, due to their sensitivity and vulnerability to environmental conditions, ability in handling and ingesting small food particles. Thus, high mortality of nauplii, associated to a failure in complete development, leads to a limited survival and maintenance of copepod populations (Chinnery and Williams, 2004). Plankton, particularly zooplankton, is considered to be a good indicator of environmental changes (Bonnet and Frid, 2004). Because they play an important role in marine and freshwater food webs, the high sensitivity of zooplankton assemblages to physical and chemical variations can be manifested as changes in abundance and community structure (Lam-Hoai et al., 2006; Porri et al., 2007). Thus, information about earlier stages is crucial to compare the ecology of the various taxa and structural patterns.

Therefore, the aim of the present work is focused on the small-sized zooplankton fraction and early development stages mainly of copepods in order to: 1) describe the spatio-temporal patterns; 2) compare distribution and structure in both arms of the estuary and 3) identify the major environmental factors that may affect the distribution of small-sized fraction of zooplankton assemblages in Mondego estuary.

MATERIALS AND METHODS

Study area

The Mondego estuary is located in a warm temperate region, on the Atlantic coast of Portugal (40º08’ N, 8º50’ W) covering an area of 3.4 km^2. It is divided in two arms with different hydrographic characteristics, separated by the Murraceira island (Fig. 1). The north arm is deeper (4-8 m during high tide, tidal range 1-3 m), mainly influenced by freshwater discharges of the Mondego river, constituting the principal navigation channel and the location of the Figueira da Foz harbour. The south arm is shallower (2-4 m during high tide, tidal range 1-3 m) and the water circulation is mainly due to tidal cycles. The freshwater input flow is low deriving mainly from a tributary, the Pranto river, which is controlled by a sluice according to rice crops irrigation needs in Mondego Valley (Lillebø et al., 2005; Marques et al., 2003). Anthropogenic activities in the estuary led to an ongoing process of eutrophication since the end of the 1980s which led to a decline of the environmental quality of the estuary, mainly in the southern arm (Cardoso et al., 2008; Chainho et al., 2007; Dolbeth et al., 2008). A management programme has been implemented since 1998 and the system is gradually recovering (Cardoso et al., 2008; Martinho et al., 2007).

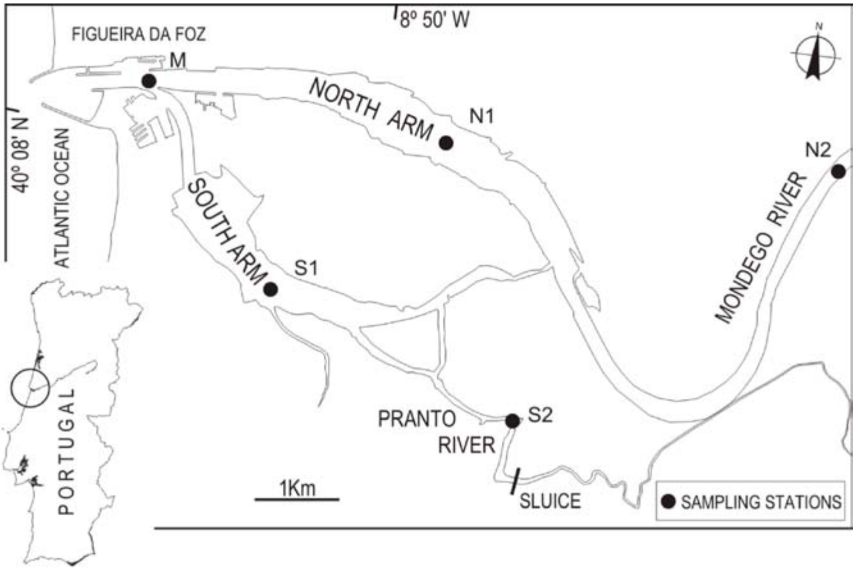

Figure 1 - Map of the Mondego estuary, located on the western coast of Portugal. Sampling stations are indicated (M – mouth station, N1 and N2 – northern arm stations, S1 and S2 – southern arm stations).

Sampling procedure and *in situ* measurements

Samples were taken monthly during high tide from February 2005 to July 2006 at five stations spread over both arms of the estuary: M – mouth station; N1 and N2 - northern arm stations; S1 and S2 – southern arm stations, in order to have representative area coverage of the system, ranging from freshwater to marine waters (Fig.1). Samples were collected by sub-surface tows (1m below the surface) with a 63 µm mesh net (mouth diameter 0.30 m), equipped with a Hydro-Bios flowmeter mounted in the mouth to estimate the volume of water filtered by the net. Sampling was performed at spring tides with few exceptions due to logistic constrains. After collection, samples were fixed in 4% buffered formaldehyde.

Simultaneously, environmental parameters such as water temperature (ºC), salinity, dissolved oxygen (DO, mg.L^{-1}), pH were measured *in situ* with appropriate sensors (WTW) and transparency with a Secchi disc (m). Simultaneously, water samples were taken in each sampling station for determination in laboratory of chlorophyll *a* (Chl *a*, mg.m^{-3}), nutrient concentrations (nitrates, phosphates, ammonia and soluble silica – mg.L^{-1}) and total suspended solids (TSS, mg.L^{-1}). Samples were filtered using a GF/C and stored frozen at -18 ºC until further analysis following standard protocols. Monthly precipitation values were measured at the Soure 13 F/01G station and acquired from INAG – Portuguese Water Institute (http://snirh.inag.pt). Annual precipitation average from 1961 to 1990 was 1016 mm that can be considered as the normal climate (IM – Portuguese Weather Institute, http://web.meteo.pt). During the study period precipitation was lower (2005-2006: 855.9mm and 2006-2007: 866.5mm). The study period can thus be considered as rather dry. (Fig. 2).

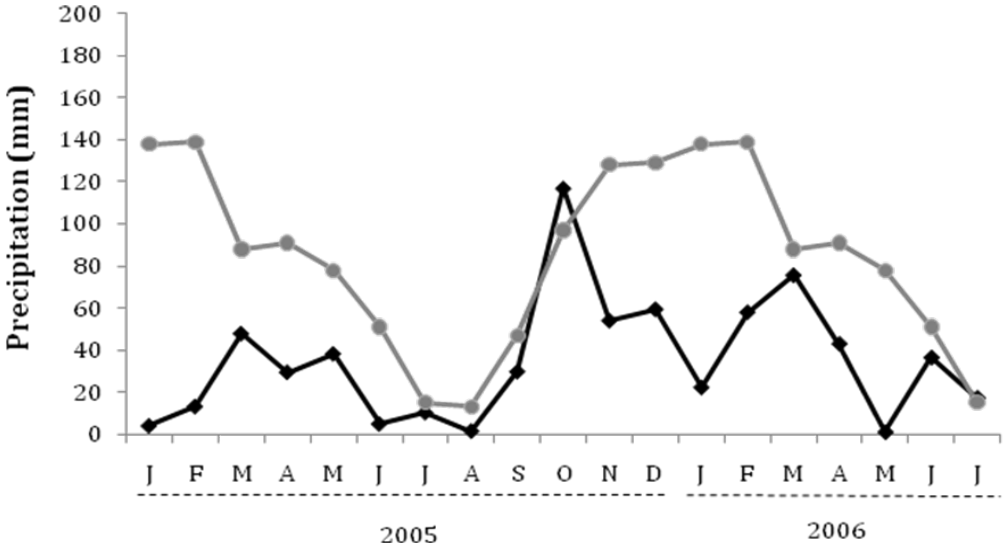

Figure 2 – Monthly precipitation (mm) in Mondego estuary during the study period (2005-2006). Grey filled circles represent monthly average of 1961 to 1990 (http://snirh.inag.pt).

Laboratory analyses

Standard methods were followed to determine nutrient concentrations (Limnologisk Metodik, 1992 for ammonia and phosphate; Strickland and Parsons, 1972 for nitrates and nitrites). Chl *a* concentration was determined by filtering 500-1000 mL of water samples volume 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). Total suspended solids (TSS) was estimated by filtering 500-1000 mL water through pre-combusted and pre-weighted Whatman GF/C filters, and dried at 60 ºC for 72 h and combusted at 450ºC for 8h (APHA, 1995). Zooplankton sub-samples were obtained for numerical abundance using a Folsom plankton splitter. The organisms were counted (number of individuals m^{-3}) and identified to the lowest possible taxon. At each subsample a minimum of 500 individuals were counted.

Data analysis

A spatial and seasonal variation analysis was performed for the five sampling stations in order to define ecological distinct groups in zooplankton assemblages, their contribution to zooplankton abundance and differences in community composition.

A Detrended Correspondence Analysis (DCA) was performed followed by a Redundancy Data Analysis (RDA) to investigate the relationship between species distribution and environmental data, using CANOCO version 4.5 (ter Braak and Smilauer, 1998). A forward selection of environmental variables was used to select those most closely associated with the spatio-temporal structure of the zooplankton taxa, and to quantify their relative importance. The statistical significance was tested with Monte Carlo permutation tests (499 unrestricted permutations) ($P < 0.05$).

Two distinct zooplankton matrixes were constructed – Copepoda and *Other Taxa* - and only the species with densities up to 3 $ind.m^{-3}$ in each sampling station were considered (Marques et al., 2007a). This cut-off was applied to eliminate those taxa having a low occurrence and decrease the number of zeros in the analyses (Beaugrand et al., 2000; Marques et al., 2007a). An (ln+1) transformation of the data was used due to the huge differences of densities between species. Environmental parameters were standardised before statistical analysis. The diversity of zooplankton assemblages in the five stations of the system was assessed as species richness (the number of species estimated for each station) and by the Shannon-Wiener index (log base 2) (Zar, 1996).

RESULTS

Environmental parameters

In table 1 the hydrological data are listed. Nutrient concentrations showed higher values associated with the upstream stations (S2 and N2). The peak of nitrates (1.84 mg.L^{-1}) and silica (2.71 mg.L^{-1}) concentrations were observed at station N2 in winter months (December, January and February). Ammonia concentrations were higher at station S2 in spring (March, April and May - 0.42 mg.L^{-1}) and summer (June, July and August) months (0.55 mg.L^{-1}). The maximum values of nitrites and Chl *a* concentration were observed in the upstream stations (S2 and N2), with the upstream station of south arm (S2) registering higher values (0.06 mg.L^{-1} and 19.42 mg.L^{-1}). For nitrates and ammonia the concentrations were higher also in winter months at all sampling stations (St M: 0.80 mg.L^{-1} (NO_3) and 0.06 mg.L^{-1} (NH_4); St N1: 1.12 mg.L^{-1} (NO_3) and 0.23 mg.L^{-1} (NH_4); St N2: 1.84 mg.L^{-1} (NO_3) and 0.25 mg.L^{-1} (NH_4); St S1: 0.41 mg.L^{-1} (NO_3) and 0.06 mg.L^{-1} (NH_4); St S2 (0.66 mg.L^{-1} (NO_3)). At station S2 the highest value of ammonia was registered in spring (0.42 mg.L^{-1}) and summer months (0.55 mg.L^{-1}). During the study period phosphate concentrations were very low at all sampling stations with the maximum values registered at station S1 in spring months (0.23 mg.L^{-1} (spring 2005) and 0.80 mg.L^{-1} (spring 2006)). Considering Chl *a* concentrations, a slight increase in spring and summer months at downstream stations (M, N1 and S1) and a considerably rise at upstream stations (S2 and N2) were observed. Salinity was high in downstream stations (M, S1 and N1), varying between 31.6 and 34.2. The highest values were recorded in spring and summer months. The lowest salinity values were observed at station N2 where the maximum and the minimum values were found in summer and winter months of 2006 ranging between 14.6 and 0.0, respectively. The water temperature varied from 9 to 25 ºC, reaching maximum and minimum values during summer and winter months, respectively. Upstream stations (N2 and S2) were characterized by the highest and lowest temperatures. Considering dissolved oxygen (DO), values ranged from 4.6 to 12.9 mg.L^{-1}. The station S2 presented the lowest values in the summer months of 2006 and the mouth station (M) the highest values in winter months of 2005 (Feb05). pH varied from 7.2 to 8.6 with the lowest value at station N1, in winter months of 2005 (Feb 05) and the highest value at station N2, in spring months of 2006. Total suspended solids (TSS) increased from lower to upper estuary at the south arm. In general, TSS increases while Secchi transparency decreases with increasing distance from the mouth.

Table 1 – Average (AVG) and standard deviation (SD) of environmental parameters measured at the sampling stations (M, N1, N2, S1 and S2) in Mondego estuary during the study period.

			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.L^{-1})**	**SST (mg.L^{-1})**	**pH**	**O_2 (mg.L^{-1})**	**Temp (ºC)**	**Sal (‰)**	**Transpar. (m)**
M	**W05 (Feb05)**	AVG	0.22	0.02	0.01	0.34	0.01	4.42	24.76	8.23	12.87	11.70	31.60	1.80
		SD	-	-	-	-	-	-	-	-	-	-	-	-
	SP05	AVG	0.18	0.01	0.01	0.10	0.05	3.84	27.24	7.93	9.56	16.47	31.53	2.40
		SD	0.04	0.01	0.00	0.07	0.03	1.38	3.01	0.56	0.78	1.17	4.06	0.85
	S05	AVG	0.70	0.01	0.00	0.07	0.01	5.76	23.42	7.87	8.33	18.40	27.30	2.57
		SD	0.59	0.01	0.00	0.05	0.01	2.99	0.50	0.32	1.22	2.88	6.45	1.12
	A05	AVG	0.18	0.01	0.00	0.05	0.00	2.97	25.80	7.94	8.88	16.03	22.97	1.47
		SD	0.09	0.01	0.00	0.05	0.00	0.99	4.66	0.34	1.43	1.94	4.45	0.15
	W06	AVG	1.42	0.03	0.01	0.80	0.06	2.02	17.51	7.84	11.00	10.97	14.87	1.40
		SD	0.31	0.00	0.00	0.15	0.03	0.67	3.97	0.32	0.34	0.35	9.99	0.36
	SP06	AVG	1.22	0.03	0.01	0.46	0.04	2.72	15.66	8.19	9.22	16.17	20.00	2.10
		SD	1.11	0.01	0.01	0.43	0.03	0.51	7.67	0.14	0.55	1.27	16.50	0.17
	S06 (June 06)	AVG	0.50	0.03	0.01	0.26	0.05	1.14	22.31	8.14	8.93	15.80	34.20	2.20
		SD	-	-	-	-	-	-	-	-	-	-	-	-
N1	**W05 (Feb05)**	AVG	0.19	0.03	0.01	0.35	0.23	2.86	24.84	8.25	10.89	12.00	33.40	1.70
		SD	-	-	-	-	-	-	-	-	-	-	-	-
	SP05	AVG	0.31	0.01	0.01	0.18	0.06	4.81	31.30	8.14	9.48	15.87	33.30	1.77
		SD	0.11	0.01	0.01	0.07	0.06	2.39	2.83	0.21	1.09	0.87	2.08	1.10
	S05	AVG	0.61	0.01	0.00	0.06	0.02	4.23	27.11	7.31	7.80	20.43	19.70	1.47
		SD	0.35	0.01	0.00	0.09	0.01	0.46	4.06	1.08	1.01	4.06	12.66	0.64
	A05	AVG	0.36	0.02	0.01	0.09	0.02	2.68	22.70	8.04	7.76	16.43	10.37	1.17
		SD	0.20	0.01	0.00	0.06	0.01	0.57	10.11	0.10	0.66	3.43	6.88	0.25
	W06	AVG	1.91	0.04	0.02	1.12	0.11	4.38	13.05	8.06	10.36	10.73	9.60	0.93
		SD	0.88	0.01	0.01	0.54	0.08	4.60	7.63	0.46	0.53	0.42	13.39	0.12
	SP06	AVG	0.73	0.04	0.01	0.41	0.06	3.32	18.82	8.16	9.35	16.13	21.43	1.40
		SD	0.57	0.01	0.01	0.45	0.06	0.21	5.78	0.09	0.74	1.63	18.34	0.66
	S06 (June 06)	AVG	0.34	0.04	0.01	0.16	0.03	8.12	28.87	8.12	8.75	16.70	32.80	2.00
		SD	-	-	-	-	-	-	-	-	-	-	-	-
N2	**W05 (Feb05)**	AVG	0.91	0.07	0.04	1.84	0.25	7.46	10.74	8.19	10.63	11.50	4.60	0.90
		SD	-	-	-	-	-	-	-	-	-	-	-	-
	SP05	AVG	1.24	0.07	0.03	0.89	0.16	10.48	19.94	7.83	7.36	19.30	9.90	1.07
		SD	0.17	0.01	0.02	0.17	0.06	6.14	5.77	0.26	1.52	3.87	7.86	0.51
	S05	AVG	1.07	0.04	0.01	0.21	0.05	10.17	22.09	7.22	6.54	23.80	9.97	0.80
		SD	0.56	0.01	0.00	0.08	0.06	4.20	0.71	0.68	1.99	1.47	13.90	0.35
	A05	AVG	1.09	0.05	0.02	0.56	0.11	3.15	23.46	7.70	7.25	17.37	1.07	0.83
		SD	0.95	0.02	0.01	0.52	0.06	1.25	5.51	0.32	0.88	4.35	1.00	0.15
	W06	AVG	2.71	0.03	0.03	1.53	0.15	8.13	7.20	8.01	9.90	10.10	0.00	0.83
		SD	0.04	0.02	0.01	0.24	0.13	6.27	3.22	0.17	0.68	0.70	0.00	0.15
	SP06	AVG	2.58	0.04	0.02	1.38	0.07	11.53	7.97	8.61	8.61	17.80	3.60	0.90
		SD	0.83	0.01	0.00	0.11	0.06	1.16	0.25	0.26	0.26	3.40	5.98	0.30
	S06 (June 06)	AVG	1.78	0.07	0.02	1.08	0.07	6.49	13.80	7.74	6.74	22.90	14.60	1.60
		SD	-	-	-	-	-	-	-	-	-	-	-	-
S1	**W05 (Feb05)**	AVG	0.29	0.03	0.01	0.41	0.01	4.42	22.29	8.21	11.01	11.80	31.70	1.60
		SD	-	-	-	-	-	-	-	-	-	-	-	-
	SP05	AVG	0.23	0.23	0.01	0.11	0.05	4.07	36.59	8.12	9.63	16.07	33.40	1.57
		SD	0.09	0.09	0.01	0.03	0.02	2.05	10.38	0.25	1.06	0.87	1.30	0.59
	S05	AVG	0.43	0.01	0.00	0.06	0.03	5.57	28.33	7.73	7.88	19.10	31.20	1.20
		SD	0.21	0.01	0.00	0.06	0.01	2.32	2.96	0.18	1.14	1.97	2.51	0.75
	A05	AVG	0.41	0.02	0.01	0.10	0.02	3.49	27.68	7.80	8.16	15.80	30.50	0.77
		SD	0.17	0.01	0.00	0.06	0.02	1.24	3.57	0.21	0.93	1.56	3.51	0.06
	W06	AVG	0.99	0.03	0.01	0.54	0.06	1.86	23.42	7.88	10.48	11.30	21.37	1.20
		SD	0.41	0.01	0.00	0.18	0.03	0.13	1.49	0.12	0.37	0.00	4.86	0.26
	SP06	AVG	0.80	0.80	0.01	0.36	0.05	3.79	19.61	8.16	9.66	17.03	27.97	1.43
		SD	0.76	0.76	0.01	0.37	0.06	1.99	3.25	0.09	0.54	0.97	8.81	0.40
	S06 (June 06)	AVG	0.41	0.03	0.01	0.18	0.05	1.34	27.11	8.08	8.75	17.20	32.80	1.50
		SD	-	-	-	-	-	-	-	-	-	-	-	-
S2	**W05 (Feb05)**	AVG	0.69	0.04	0.02	0.26	0.05	16.12	33.22	8.11	9.65	12.50	26.10	0.60
		SD	-	-	-	-	-	-	-	-	-	-	-	-
	SP05	AVG	0.97	0.06	0.03	0.15	0.42	8.13	49.71	7.92	8.05	20.77	28.10	0.57
		SD	0.25	0.01	0.01	0.07	0.08	3.91	19.33	0.07	0.21	4.09	1.73	0.12
	S05	AVG	1.40	0.06	0.03	0.04	0.29	19.42	37.59	7.55	6.29	24.83	18.93	0.47
		SD	0.83	0.02	0.02	0.01	0.05	11.55	6.97	0.31	1.78	1.75	15.45	0.06
	A05	AVG	0.87	0.06	0.06	0.12	0.30	19.29	45.09	7.50	6.50	17.50	30.53	0.53
		SD	0.05	0.01	0.01	0.02	0.06	10.60	12.40	0.44	1.14	3.55	3.97	0.06
	W06	AVG	1.79	0.04	0.04	0.66	0.30	5.52	28.05	7.58	9.23	8.83	11.20	0.60
		SD	0.62	0.01	0.01	0.26	0.16	1.11	8.17	0.34	0.99	2.02	8.45	0.10
	SP06	AVG	1.85	0.05	0.05	0.48	0.34	11.03	27.02	6.75	7.83	20.87	17.33	0.57
		SD	0.97	0.02	0.02	0.48	0.09	2.56	2.84	0.42	0.08	1.01	6.86	0.12
	S06 (June 06)	AVG	1.72	0.05	0.04	0.13	0.55	6.44	26.91	7.51	4.63	23.20	24.00	0.60
		SD	-	-	-	-	-	-	-	-	-	-	-	-

Note: Winter 2005 (W05) means February 2005 and Winter 2006 (W06) means December of 2005, January and February 2006; Spring 2005 (SP05) and Spring 2006 (SP 06) means March, April and May months of 2005 and 2006 correspondingly; Summer 2005 (S05) and Summer 2006 (S 06) means June, July and August months of 2005 and 2006 respectively; Autumn 2005 (A05) means September, October and November months of 2005.

Zooplankton abundance, composition and distribution

In table 2 are referred the main groups identified in this study. Life-history stages are reported, mainly to Copepoda, where were identified naupliar, juveniles and adults stages. Figure 3 shows no distinct seasonal pattern in the distribution of Copepoda development stage groups. However they appeared to be numerically less important during winter months. Nauplii of copepod were absent at the south arm in March and the adults were absent in the northern arm at station N1 in December 2005 and April 2006, as well as at the south arm at station S2 in August 2005 (Fig. 3A). Additionally, at times of higher precipitation (see Fig. 2 e.g. October 2005) nauplii of copepods were observed in higher abundance (total abundance: 1.84×10^{6} ind.m^{-3}) at the mouth station (St M). At this station no data was obtained in April 2005 due to logistic problems.

The zooplanktonic groups with lowest abundance (‘*Other Taxa*’, Fig. 3B) included: Cladocera, ichthyoplankton, Medusae, Mysidaceae and larvae of Decapoda, Polychaeta, Oligochaeta and Cephalopoda. For *Other Taxa* a seasonal pattern was registered. In general, higher abundance was recorder in summer months. Together, these taxonomic groups presented lower total abundance ($4.28 \times 10^{2} \pm 7.44 \times 10^{3}$ ind.m^{-3}) than the copepod species ($7.30 \times 10^{3} \pm 3.99 \times 10^{5}$ ind.m^{-3}). At the north arm of the estuary, *Other Taxa* zooplankton exhibited the highest abundances in summer/autumn 2005 ($3.23 \times 10^{2} \pm 1.24 \times 10^{3}$ ind.m^{-3}/ $5.86 \times 10^{2} \pm 2.19 \times 10^{3}$ ind.m^{-3}) followed by a decrease of abundance in winter 2006 ($27 \pm 1.40 \times 10^{2}$ ind.m^{-3}), rising again in spring 2006 ($2.92 \times 10^{2} \pm 1.65 \times 10^{3}$ ind.m^{-3}) (Fig. 3B). Despite the lowest abundance (2 ± 15 ind.m^{-3}) observed during winter 2005 at the stations of the southern arm, the *Other Taxa* groups were more abundant in later autumn/winter (November/December at station S1: $1.07 \times 10^{3} \pm 4.02 \times 10^{3}$ ind.m^{-3} and October/November at station S2: $6.89 \times 10^{2} \pm 4.52 \times 10^{3}$ ind.m^{-3}) in this area of the estuary.

Table 2 – List of taxonomic groups and codes used in RDA analysis.

	Taxon	**Code**	**Code nº (RDA analysis)**
Copepoda	Nauplii Copepoda	Naco	✦
	Copepodite / Adult *Euterpina acutifrons*	CpEuac / Euac	■
	Copepodite / Adult *Paronychocamptus nanus*	CpPana / Pana	□
	Copepodite / Adult *Microsetella norvegica*	CpMino / Mino	□
	Copepodite / Adult *Microsetella* sp.	CpMisp /Misp	□
	Copepodite *Canuella* sp.	CpCansp	□
	Copepodite *Harpacticus* sp.	CpHarsp	□
	Macrosetella gracilis	Macgr	□

	Paraleptastacus cfr. *spinicauda*	Parspi	□
	Mesochra sp.	Mesp	□
	Metis cfr. *ígnea*	Metig	□
	Ectinosoma melaniceps	Ectmel	□
	Ectinosoma sp.	Ectsp	□
	Nannopus palustris	Napa	□
	Harpacticus obscurus	Harob	□
	Leptocaris brevicornis	Lepbr	□
	Apodopsyllus sp.	Aposp	□
	Copepodite *Acartia* sp.	CpA	△
	Copepodite / Adult *Acartia clausi*	CpAcl / Acl	▲
	Copepodite / Adult *Acartia tonsa*	CpAto / Ato	▶
	Copepodite / Adult *Centropages* sp.	CpC / Cesp	△
	Copepodite / Adult *Centropages typicus*	CpCty / Cety	△
	Copepodite /Adult *Centropages chierchiae*	CpCch /Cech	△
	Copepodite *Pseudoparaclausocalanus* (*Paracalanus* type-form)	CpPspacl	△
	Copepodite / Adult *Pseudocalanus elongatus*	CpPsel /Psel	△
	Copepodite *Pseudocalanus* sp.	CpPs	△
	Copepodite *Pseudocalanus parvus*	CpPpa	△
	Copepodite *Paracalanus* sp.	CpP	△
	Paracalanus parvus	Papa	△
	Copepodite / Adult *Clausocalanus arcuicornis*	CpClar /Clar	△
	Copepodite *Clausocalanus* sp.	CpCl	△
	Copepodite / Adult *Calanus helgolandicus*	CpChe / Cahe	△
	Copepodite / Adult *Temora longicornis*	CpTlon / Telo	△
	Copepodite / Adult *Eurytemora velox*	CpEuve / Euve	▲
	Copepodite *Eurytemora* sp.	CpEu	△
	Copepodite / Adult *Copidodiaptomus numidicus*	CpConu / Conu	▽
	Copepodite / Adult C*alanipeda aquae dulcis*	CpCaqdu / Caqudu	▼
	Copepodite / Adult *Isias clavipes*	CpIscl / Iscl	△
	Copepodite Calanoida	CpCal	△
	Copepodite *Oithona* spp.	CpOi	○
	Copepodite / Adult *Oithona plumifera*	CpOipl / Oipl	○
	Copepodite / Adult *Oithona nana*	CpOina / Oina	●
	Copepodite / Adult *Oithona helgolandica*	CpOihe / Oihe	○
	Copepodite / Adult *Acanthocyclops robustus*	CpAcro / Acro	●
	Copepodite *Acanthocyclops* sp.	CpAc	○
	Copepodite / Adult Cyclopoida	CpCyc / Cyc	○
	Cyclopidae	Cyae	○
	Copepodite /Adult *Corycaeus anglicus*	CpCoan / Coan	◇
	Copepodite *Corycaeus* sp.	CpCo	◇
	Copepodite / Adult *Oncaea* sp.	CpOn / Onsp	◇
	Sapphirina sp.	Sap	◇
	Pachos punctatum	Papu	◇
	Copepodite not identified	Cpni	×
Cirripedia	Cypris Cirripedia	Cyci	□
	Nauplii Cirripedia	Naci	□
Cladocera	*Daphnia longispina*	Dplo	△
	Daphnia sp.	Dasp	△
	Bosmina longirostris	Boslo	△
	Podon polyphemoides	Popo	△
	Podon leuckartii	Pole	△
	Evadne nordmanni	Evnor	△
	Penilia avirostris	Peav	△
Mollusca	Veligera Gastropoda	VeG	○
	Post-Veligera Gastropoda	PosVeG	○
	Veligera Bivalvia	VeB	○

	Post-Veligera Bivalvia	PosVeB	○
	Cephalopoda (larvae)	LaCep	○
	Cephalopoda	Cep	○
Polychaeta	Terebellidae (larvae)	Terla	◇
	Syllidae-Autolytus sp. (larvae)	SyAusp	◇
	Polychaeta (larvae)	Pola	◇
	Trocophora Polychaeta	Tropol	◇
	Eunicidae (larvae)	Eula	◇
	Spionidae (larvae)	Sptro	◇
	Magelonidae (larvae)	TroMa	◇
Siphonophora	*Diphyes* sp.	Dy	✦
	Muggiaea sp.	Musp	✦
	Muggiaea atlantica	Muat	✦
Medusae	Cnidaria (Medusae)	Cni	×
	Lizzia blondina	Lizbl	×
	Obelia sp.	Ob	×
	Solmaris corona	Soco	×
	Sarsia prolifera	Sapr	×
	Podocoryne minima	Pomi	×
	Zanclea costata	Zaco	×
	Liriope tetraphylla	Lite	×
Mysidaceae	Mysidaceae not identified	Myni	■
	Neomysis integer	Nein	■
	Heteromysis formosa	Hefo	■
	Gastrosaccus spinifer	Gasp	■
Euphausiacea	Euphausiacea (larvae)	Eup	+
Decapoda	Decapoda not identified	Deni	▲
	Zoea Processa sp.	Pro	▲
	Zoea Upogebia sp.	Upo	▲
	Zoea Palaemon sp.	Pal	▲
	Zoea Crangon crangon	Crcr	▲
	Zoea Carcinus maenas	ZoCama	▲
	Zoea Pachygrapsus marmoratus	ZoPama	▲
	Zoea Pisidia longicornis	ZoPilo	▲
Appendicularia	*Oikopleura dioica*	Oidi	▶
	Fritillaria borealis	Frbo	▶
Chaetognatha	*Sagitta friderici*	Safr	▲
Ichthyoplankton	Fish larvae	Fila	▽
Echinodermata	*Ophiopluteus* sp.	Ophi	▼
Isopoda	*Eurydice spinigera*	Eusp	●
	Paragnathia formica	Pafo	●
	Isopoda (larva not identified)	Isolani	●
Amphipoda	*Gammarus* sp.	Gam	+
	Ostracoda	Ost	●
	Acaridae	Aca	□
	Aranidae	Ara	■
	Larva not identified	lani	×
	Organism not identified	Orgni	◆

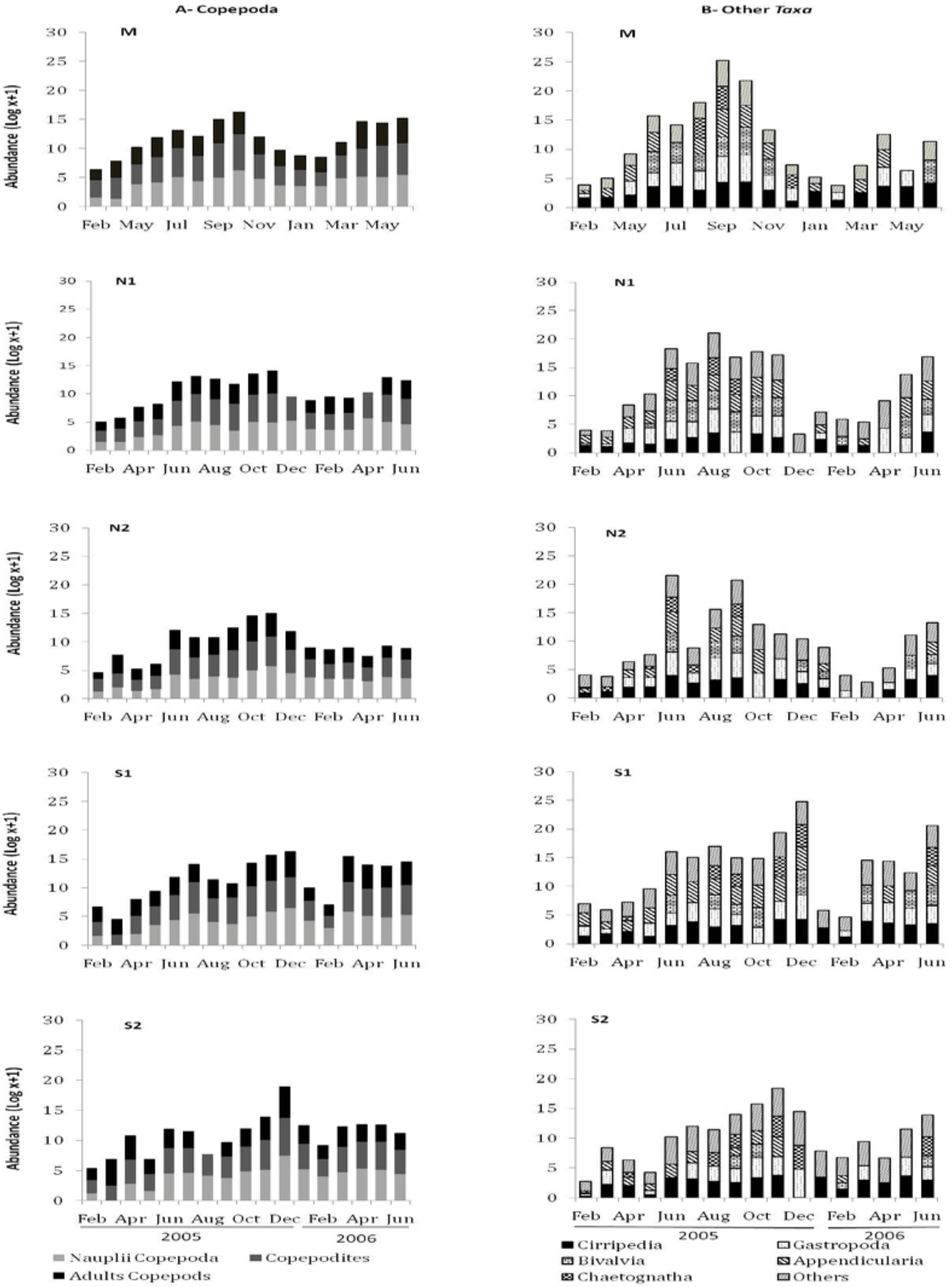

Figure 3 – Monthly pattern of the abundance (ind.m^{-3}) of main zooplankton groups (A) Copepoda and (B) *Other* T*axa* at each station. Logistic problems did not allow collecting the samples at station M at April 2005.

During the study period, Chaetognatha occurred regularly in the last semester of 2005 at downstream stations (M, N1 and S1) and sporadically in station N2. Appendicularia (*Oikopleura dioica* Fol, 1872 and *Fritillaria borealis* Lohman, 1896) were present in all sampling stations, being more abundant in the first year of the study period ($1.20\times10^3 \pm 4.92\times10^3$ ind.m^{-3} at 2005; $1.40\times10^2 \pm 4.91\times10^2$ ind.m^{-3} at 2006) and in downstream stations (M: $2.76\times10^3 \pm 1.02\times10^4$ ind.m^{-3}, S1: $3.92\times10^2 \pm 8.04\times10^2$ ind.m^{-3} and N1: $1.99\times10^3 \pm 3.96\times10^3$ ind.m^{-3}). Bivalvia larvae presented higher abundances in autumn 2005 at the mouth station (M: $8.08\times10^3 \pm 1.12\times10^4$ ind.m^{-3}) and in winter 2006 at upstream station of south arm (S1: $4.39\times10^3 \pm 6.21\times10^3$ ind.m^{-3}). In general, Cirripedia (e.g. Cypris and nauplii) and Gastropoda larvae occurred regularly in both arms of the estuary, with the latter one being more abundant ($1.48\times10^4 \pm 1.82\times10^4$ ind.m^{-3}) in autumn at the mouth station (M).

Concerning the main groups of zooplankton, copepods were the most abundant group with 95.6% of the total abundance followed by Gastropoda, Cirripedia, Bivalvia and Appendicularia representing 0.6%, 0.4%, 0.2% and 0.2%, respectively (Fig. 4B). A set of groups with lowest abundances ("Others") reached 3% of the total abundance and included the following zooplankton groups: Chaetognatha, Cladocera, ichthyoplankton, Medusae, Mysidaceae, larvae of Decapoda, Polychaeta, Oligochaeta and Cephalopoda. A total of 112 zooplankton taxa were recorded. In general, the number of species at station N2 was higher with highest values in May of 2005. The sampling stations of the north arm (N1 and N2) and the mouth station (M) showed a higher number of species than the southern arm, with the upstream station (S2) showing an irregular pattern. Analyzing the Shannon-Wiener index (H'), in figure 5, higher values were found at stations of the north arm (N1 and N2) than in the south, with the former exhibiting the highest value (max: 4.19). A decrease in heterogeneity values from March to December 2005 at station N1 was recorded, followed by a decrease in the number of species. During the study period it was not possible to recognize a trend between sampling stations. Still, it was observed a decrease in heterogeneity and in the number of species at the five sampling stations. This may be due to the huge variety of species and stages of small zooplanktonic species present in the samples. Some of them occurred in very high densities (e.g. nauplii Copepoda and nauplii Cirripedia) in contrast to species that appear only once or twice during the year. Figure 6 shows the temporal distribution at each sampling station of the main zooplankton species. Copepods, mainly the first stages (nauplii and copepodites), were clearly dominant along the estuary (except station M). This group showed a rise of abundance from February 2005 to November 2005/December 2005, with nauplii of copepod and copepodites exhibiting a peak of abundance in the latter period. Copepod nauplii showed the highest abundance following cyclopoid species represented mainly by *Oithona* spp. and *Oithona plumifera* Baird, 1843.

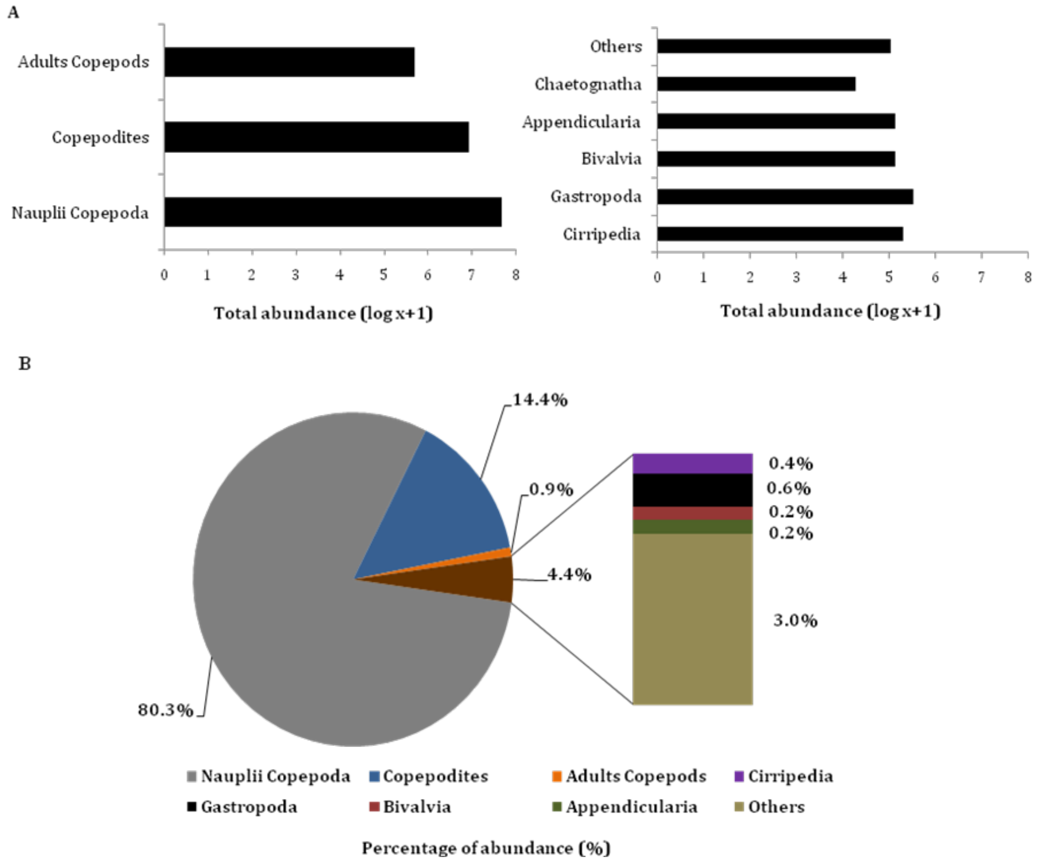

Figure 4 – (A) Total abundance [log(x+1)] and (B) relative abundance (%) of the different zooplanktonic groups to total zooplankton abundance of 63 μm taxocenosis in the Mondego estuary.

Other copepod species that occur with high frequency in Mondego estuary are copepodites and adults of *Euterpina acutifrons* Dana, 1849 (harpacticoid), copepodites of *Acartia tonsa* Dana, 1849 (calanoid), copepodites of *Acanthocyclops robustus* G. O. Sars, 1863 (cyclopoid) and copepodites of *Paracalanus*-type form (calanoid). Copepodites of *Pseudocalanus* sp., *Paracalanus* sp. and *Clausocalanus* sp. are referred as *Paracalanus*-type form when they do not show the main characteristics of each genus to their identification. Copepodites of *O. plumifera* occur in higher densities at stations of the southern arm and at the mouth station

(St S1, St S2 and St M). Cladocerans occur regularly in the estuary, with higher numbers ascribed to the marine species *Podon polyphemoides* Leuckart, 1859*,* at stations M, N2 and S1, and to the freshwater species *Bosmina longirostris* O. F. Müller, 1776 at St S2 and north arm stations (mainly at St N2). Polychaeta larvae, namely Spionidae trochophora, showed higher abundance at stations M, N1 and S2. Mollusca (Gastropoda post-veligers) also dominate the zooplankton in Mondego estuary at the upstream station of northern arm (St N2).

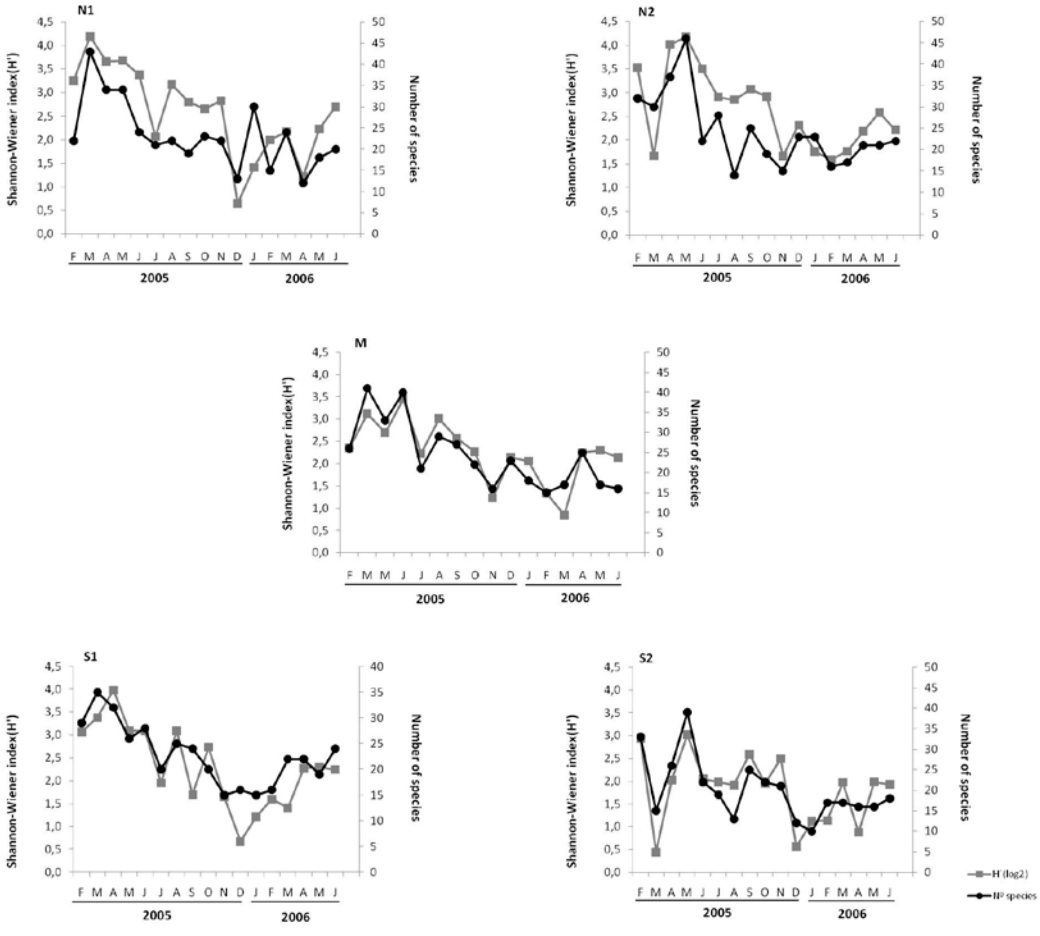

Figure 5 – Spatial and temporal variation of biodiversity based on number of taxa (dark line) and Shannon-Wiener index (grey line).

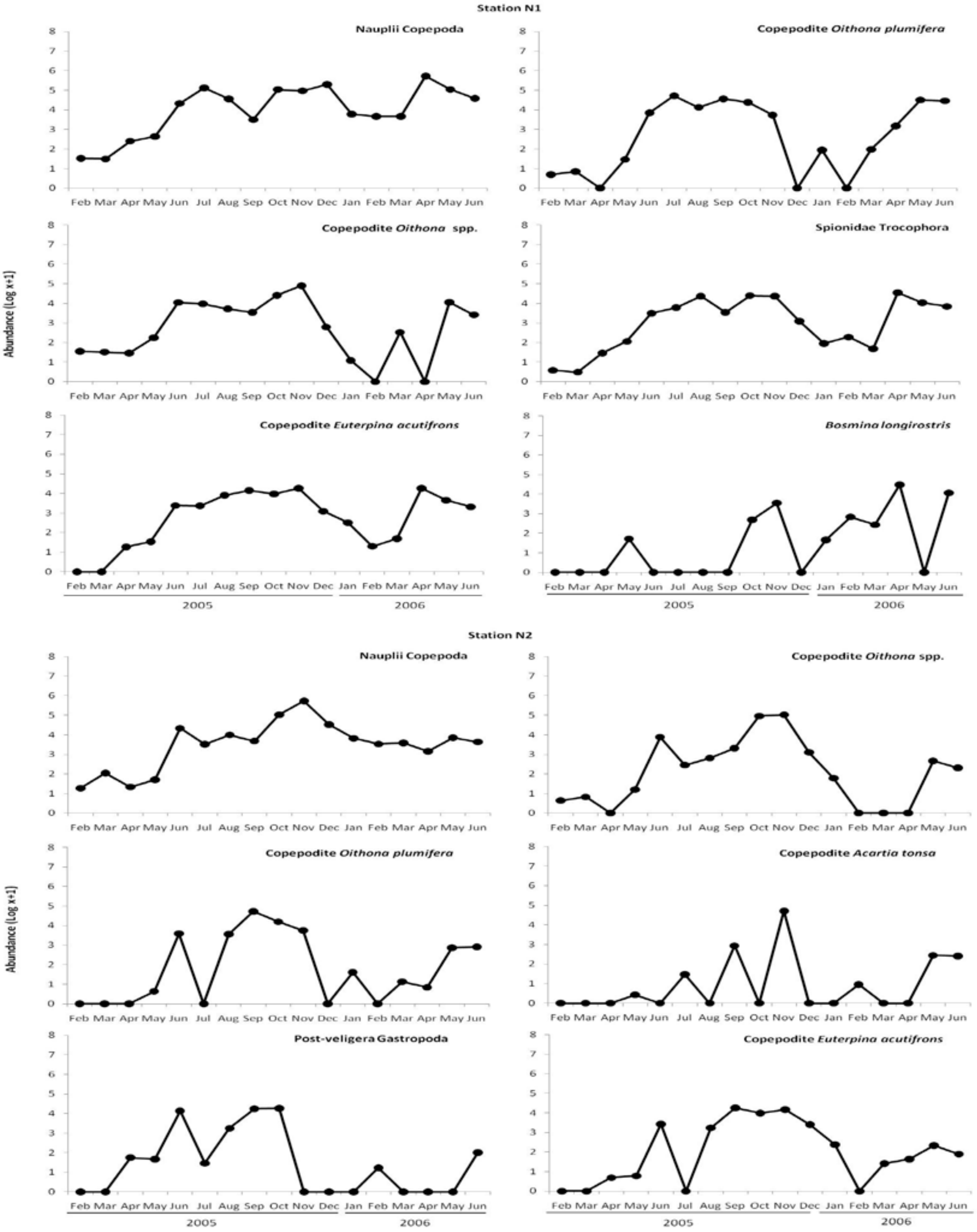

Figure 6 – Monthly pattern of abundance [log(x+1)] of the six most abundant zooplankton taxa at the five sampling station.

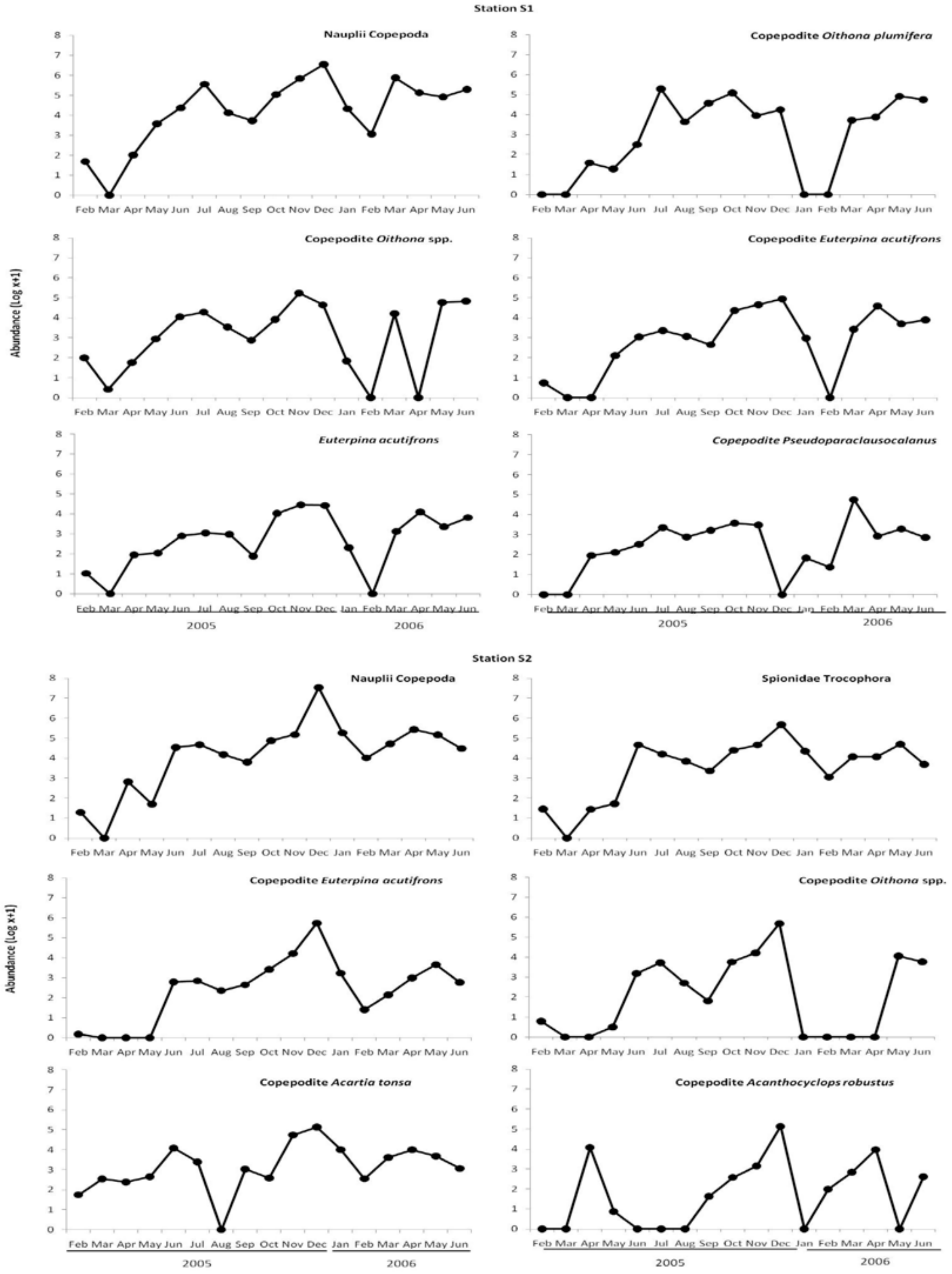

Figure 6 – [continued]

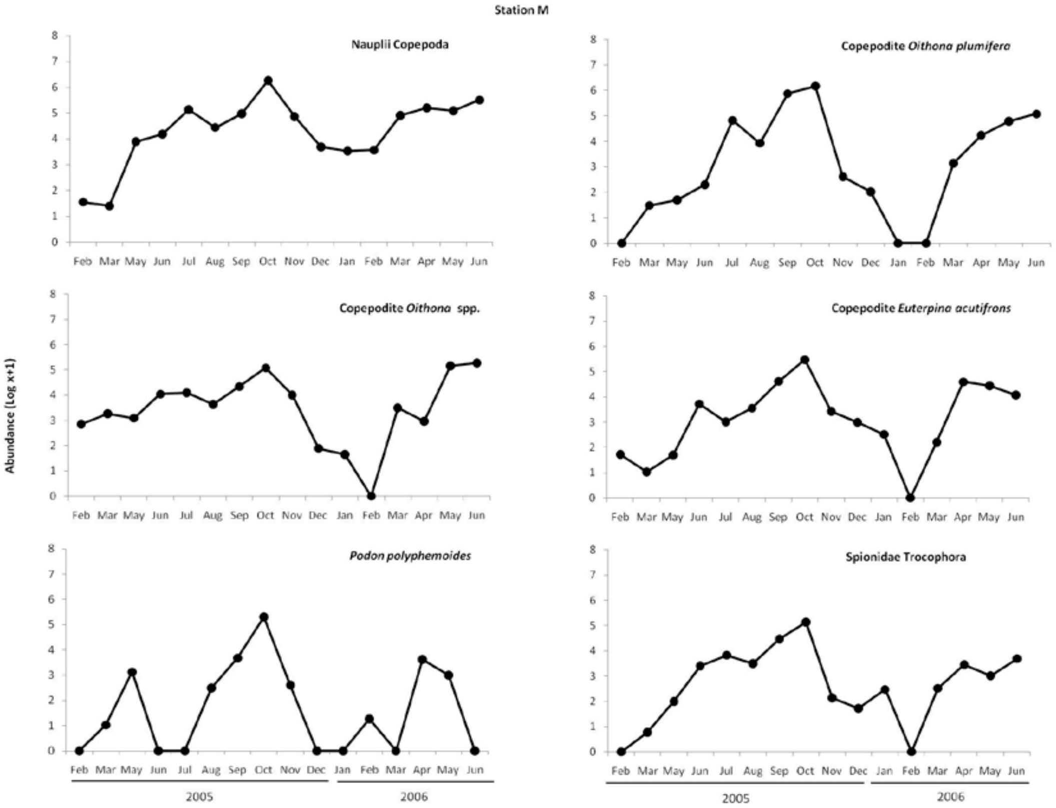

Figure 6 – [continued]

The total of inertia values from DCA analysis was <2.6 (2.5). The results of redundancy analysis of the environmental parameters and copepods and of environmental parameters and *Other Taxa* species are presented in figures 7A and 7B, respectively. Among the environmental factors, Monte Carlo permutation showed that nitrate, ammonia, temperature, total suspended solids (TSS), dissolved oxygen, nitrite and phosphate were significant in explaining the ordination, in decreasing order of importance (Fig. 7A). The environmental parameters that contributed significantly to explain the distribution in plot 7B are nitrates, salinity, ammonia, nitrite, silica and dissolved oxygen, in decreasing order of importance. The environmental variables considered in the RDA analysis explains in the first canonical axis 53.5% and 62.9% of the variance (Figs. 7A and 7B respectively), being strongly related with temperature and nitrate concentrations. Dissolved oxygen, nitrite and phospate concentrations were correlated with the second canonical axis (explaining more than 21% of the variance (Fig. 7A). At figure 7B, dissolved oxygen concentration and salinity were correlated with the second canonical axis, explaining more than 14% of the variance. Relatively to figure 7A, the first axis is positively correlated with nutrient concentrations and dissolved oxygen and negatively with temperature and TSS (Table 3A). Furthermore, second axis has higher correlation with nitrites, phosphates and dissolved oxygen (Table 3A). In figure 7B, the first axis is positively correlated with nitrate, nitrite, ammonia, DO and silica and negatively with salinity, which has an important contribution in second axis (Table 3B). The freshwater and estuarine copepods species such as copepodites of *A. robustus*, *Copidodiaptomus numidicus* Gurney, 1909, *Eurytemora velox* Lilljeborg, 1853, *Calanipeda aquae dulcis* Kritschagin, 1873 and adults and juveniles of *A. tonsa* were situated in the right side of the plot (Fig. 7A) associated with lower temperature and TSS and characterized by higher quantities of nutrients. Similarly, the Cladocera *Daphnia* sp., *Daphnia longispina* O. F. Mueller, 1785, *B. longirostris* and *Penilia avirostris* Dana, 1849 and Ostracoda inhabited the zone with lower salinity situated in the lower right side of the plot (Fig. 7B). The marine copepodites and adults like *Acartia clausi* Giesbrecht, 1889, *Calanus helgolandicus* Claus, 1863 and *Temora longicornis* O. F. Müller, 1785, the Cladocera *Podon leuckartii* G. O. Sars, 1862, *P. polyphemoides*, *Evadne nordmanni* Lovén, 1836, Decapoda (e.g. *Crangon crangon* Linnaeus, 1758, *Processa* sp.) Appendicularia (e.g. *O. dioica*), Polychaeta (e.g. Terebellidae larvae), Mollusca larvae and Hydromedusae and some gelatinous zooplankters (e.g. *Dyphies* sp., *Muggiaea* sp., *Muggiaea atlantica* Cunningham, 1892, *Lizzia blondina* Forbes, 1848) were situated in the left side of the plot, associated with high temperature, TSS and salinities, (see Figs. 7A and 7B, respectively). A standard taxonomic list with the codes of zooplankton species used is presented in table 2.

DISCUSSION

This work represents the first study of small zooplankton communities in Mondego estuary and its relationship to physical and chemical environmental forcing factors. The undersampling of the early developmental stages and smaller copepod specimens in the studies of mesozooplankton assemblages on Mondego estuary conducted so far, may have masked some seasonal trends corresponding to the reproductive activities. Temporal changes in the relative abundance of nauplii and copepodites are useful to detect recruitment and growth rate of some species of copepod which have seasonal breeding.

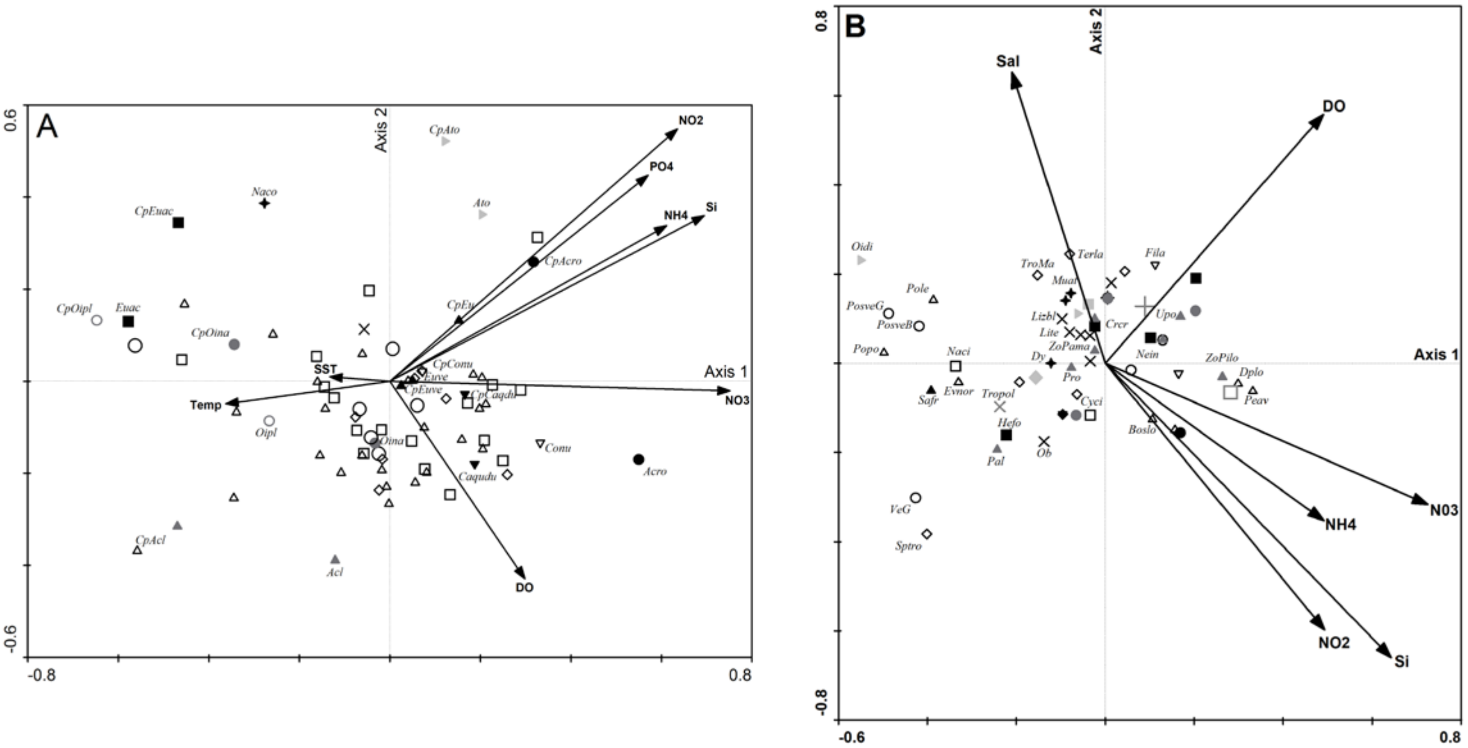

Figure 7 – Results of RDA analysis (A) Environmental variable vectors and Copepod species. Eigenvalues: Axis 1 – 0.146; Axis 2 – 0.057; Axis 3 – 0.026; Axis 4 – 0.016. Sum of eigenvalues: 0.245); (B) Environmental variable vectors and no Copepod species. Eigenvalues: Axis 1 – 0.125; Axis 2 – 0.027; Axis 3 – 0.021; Axis 4 – 0.013. Sum of eigenvalues: 0.186).

Table 3 - Biplot scores of the most significant environmental variables with Redundancy analysis (RDA) axes to environmental variable vectors and Copepod species (A) and environmental variable vectors and no Copepod species (B).

A

Name	AX 1	AX 2	AX 3	AX 4
DO	0.2984	-0.4296	-0.1656	-0.176
T	-0.3625	-0.0493	0.4521	-0.0502
Si	0.6947	0.3598	-0.4716	-0.0443
PO_4	0.5705	0.4473	0.4211	-0.1678
NO_2	0.6352	0.548	0.3544	0.2852
NO_3	0.7515	-0.0209	-0.5104	-0.014
NH_4	0.6118	0.3378	0.5012	0.1434
TSS	-0.1326	0.0096	0.7169	0.5023

B

Name	AX 1	AX 2	AX 3	AX 4
DO	0.4901	0.5582	-0.2574	-0.1902
Sal	-0.2098	0.6518	0.6765	-0.2212
Si	0.643	-0.6596	-0.1613	0.1916
NO_2	0.4927	-0.5978	0.3773	-0.2611
NO_3	0.7255	-0.3166	-0.5317	-0.2246
NH_4	0.4909	-0.3523	0.5012	0.1664

Thus, studies which did not sort the early copepod stages have no information about changes in species and thus cannot infer about seasonality fluctuations due to a lack of information about the earlier developmental stages. This frequent undersampling of small copepod species, and mainly the earlier stages, may lead to a limited view of the ecology of planktonic systems and must be considered when comparing results (Calbet et al., 2001; Turner, 2004).

As in other marine coastal areas (e.g. Mpenjati Estuary, South Africa; Ria de Aveiro, northern of Portugal) (Kibirige and Perissinotto, 2003; Leandro et al., 2007), copepods dominate the mesozooplankton in the Mondego estuary (Marques et al., 2009; Primo et al., 2009; Vieira et al., 2003). Indeed, in the Mondego estuary the total zooplankton abundance reflects the seasonal variation of the copepods population. In the mesozooplankton assemblages, Copepoda was the main dominant group and *A. tonsa* the more abundant *taxon* (Marques et al., 2007a, b; Pastorinho et al., 2003; Primo et al., 2009). The upper reaches of the northern arm were dominated by *A. robustus* and *C. numidicus* and in the southern arm the resident estuarine copepod *A. tonsa* was dominant. Marine reaches of the estuary are usually dominated by marine calanoid copepods *A. clausi*, *T. longicornis* and the cyclopoid copepod *Oithona nana* Giesbrecht, 1892 (Marques et al., 2007a; Vieira et al., 2003). The present study complemented quite well the mesozooplankton data of the Mondego estuary adding relevant new zooplanktonic information. For example, amongst small copepods, the cyclopoid *Oithona* is a key element and can be seen as the most abundant genus in the zooplankton community (Porri et al., 2007; Soussi et al., 2000). Evidence indicates that *Oithona* is being underestimated due to larger mesh net used in zooplankton sampling (Gallienne and Robin, 2001). This fact is in agreement with our results, which demonstrated that the methodology used in previous zooplankton studies underrepresented the smaller-sized copepods, particularly *O. plumifera*. This species is one of the major food sources for many fish larvae, Chaetognatha and jellyfish, which depend almost exclusively on it, making these small copepods an important element in the structure of many food webs (Hansen et al., 2004; Porri et al., 2007). This cyclopoid shows a wide distribution from south coast of South Africa (Porri et al., 2007) to south western Mediterranean and Central Baltic Sea (Hansen et al., 2004; Soussi et al., 2000). Moreover, these results emphasize the availability and abundance of other small copepods such as harpacticoids (Gonçalves et al., 2010), *Pseudocalanus* sp. and their early development stages in the water column, which have been underestimated due to the large mesh size previously used in zooplankton studies in the Mondego estuary (Azeiteiro et al., 2000; Marques et al., 2006).

The calanoid copepod *A. tonsa* reached higher abundances in upstream stations of southern arm (S2) where the concentration of particulate organic matter (TSS) was highest, a distributional pattern already reported (Murrel and Lores, 2004; Tackx et al., 2004). Moreover, and according to Ianora (1998), *A. tonsa* finds a winter benefit from the early phytoplankton bloom in terms of reproductive performance. Afterwards its annual peak in egg production rate occurs in February with increasing numbers of juveniles and adults in spring. It hatches from resting eggs in the sediments when temperatures exceed 15 ºC and due to its sensitivity to temperature one might predict an increased period of dominance of *A. tonsa* in response to warmer winters or earlier springs, in Narragansett Bay (USA) (Sullivan et al., 2007). Additionally, Holste and Peck (2006) stated that temperature and salinity are key factors in the reproductive success of *A. tonsa*, influencing the cosmopolitan distribution of this species. These authors observed the highest rate of eggs production at high temperatures (23 ºC) and salinities between 17 and 25. Adult *Acartia* congeners have distinct seasonal and spatial distribution patterns but nauplii of all species survive at higher salinities. According to Chinney and Williams (2004) the poorly swimming nauplii allows a differential distribution of different life stages in estuarine systems. At Mondego estuary, copepods, mainly the first stages (nauplii and copepodites) were clearly dominant along the estuary (except at mouth station – M) due to higher salinities. Moreover, nauplii occurred mainly at the mouth station during rainfall months, being advected from upstream areas, which corroborated the results of those authors. However, *A. tonsa* adults are less tolerant to salinity than adults of *A. clausi* (Chinnery and Williams, 2004), as found in the present study. This study clearly showed that *A. tonsa* copepodites reached higher densities in upstream stations (N2 and S2) (contrarily to *A. clausi* copepodites) in November and December and in spring months in the estuary. This distribution pattern is related to salinity and temperature which reached the lowest and highest values at upstream stations (N2 and S2), respectively. Our results are in accordance to Leandro et al. (2006) and Chinnery and Williams (2004) which stated the development time of *A. tonsa* decreases with increasing temperature. Furthermore, Chinnery and Williams (2004) claimed *A. tonsa* is mainly restricted to upper reaches of Southampton Water (UK) estuary with lower values of salinity. Moreover, as mentioned above, autumn and winter months showed the lowest values of salinity, due to precipitation, and lowest values of temperature. Considering nauplii and copepodites stages, salinity, temperature and food quality and quantity may affect their development and growth rate (Chinnery and Williams, 2004; Leandro et al., 2006). According to an experiment conducted by Fernández (1979) some calanoid naupliar stages, have different optimal size of prey due to low ability in handling and ingesting small food particles as well as due to food abundance and composition. Therefore, nauplii stages present the highest mortality (Chinnery and Williams, 2004) due to differential tolerances and physiological regulation strategies for success in adverse environments (e.g. Ozaki and Ikeda, 1997; Tester and Turner, 1991) such as estuaries. For instance, the tolerance to temperature and salinity by nauplii of calanoid copepods (e.g. *Eurytemora affinis* Poppe, 1880) is controlled by regulatory genes related to the synthesis of proteins for metabolic activity (Devreker et al., 2004). Furthermore, naupliar development duration becomes longer at lower temperatures and could not be complete below 5 ºC (Devreker et al., 2004; Roddie et al., 1984). Moreover, other nauplii are more tolerant to high salinities than adults being frequently flushed into open sea water imposing an adaption to survive at those conditions. The *E. velox* adults and copepodites were abundant and clearly associated to the marine - freshwater gradient (Fig. 7) in a brackish position. These species adults can endure freshwater and a salinity of 40, however since nauplii do not tolerate wide variation of salinity a population could not be maintained (Chinnery and Williams, 2004; Nagaraj, 1988).

The occurrence and distribution of zooplankton species in Mondego estuary, as in other marine coastal areas, are related with biotic and abiotic factors (e.g. Marques et al., 2007a, b; Turner, 2004). The main environmental gradients are related with temperature and salinity which have a pronounced effect in spatial and temporal patterns of zooplankton species (David et al., 2005; Molinero et al., 2005; Uriarte and Villate, 2005). Our data evidenced also a clear spatial temperature, salinity, gradient associated to a marine, brackish – freshwater faunal gradient. As stated by Marques et al. (2006; 2007b) the differences in zooplankton abundance between the sampling stations and, more precisely, between north and south arms are related to distinct hydrological conditions in such a way that both arms can be considered as two different subsystems. Freshwater discharges occur mainly in the north arm causing a higher salinity variation. In the southern arm salinity changes are slighter due to a more stable water column and water masses and shallow depth, added to nutrient enrichment predominantly in upstream areas enhancing higher zooplankton abundances

(Marques et al., 2007a, b). For example, *E. acutifrons* was observed in all sampling stations due to its ability to adapt to a wide range of salinity and temperature conditions presenting a wide distribution (Gonçalves et al., 2010). So species-specific distribution with generalists (e.g. *E. acutifrons*) occurring all over the estuary and specialists (e.g. *A. tonsa*) found in a restricted area.

Studies carried out by Vieira et al. (2003) stated that copepods, mainly copepodites stages, and larvae of Mollusca, Cirripedia and Polychaeta were dominant in the Mondego estuary. The same pattern was also reported from other estuarine systems (e.g. Ria Formosa, southern Portugal; Mediterranean coastal area) (Siokou-Frangou, 1996; Sprung, 1994). Similar results were obtained in the present study, where nauplii of copepod, copepodites and larvae of Mollusca, Polychaeta and Cirripedia demonstrated to be important components of the plankton in the Mondego estuary. Vieira et al. (2003) also claimed that *O. nana*, *Oithona similis* Claus, 1866, *A. tonsa* and *A. clausi* were the most common species in the Mondego estuary. In this study, *Oithona* spp., and particularly *O. plumifera*, is one of the dominant copepod species showing higher densities than reported by Vieira et al. (2003). This could be explained due to differential sampling stations in both works. The study performed by Vieira et al. (2003) was limited to the south arm of the Mondego estuary and in addition, copepodites of *Oithona* spp. may include copepodites of *O. nana*. According to Porri et al. (2007) this could be supported by physical explanations as *Oithona* spp. presents an irregular patchiness and extreme variability in abundance making the densities of this genus, and particularly *Oithona plumifera* highly unpredictable due to variable hydrodynamic conditions. Patterns of local hydrodynamics are extremely variable and change over long and short temporal scales making the distribution of zooplankton very patchy. In the present work, adults of *Oithona* spp. were typically found in downstream regions of the Mondego estuary, while copepodites occurred in all study area. Probably this occurs due to a higher sensitivity of adults of *Oithona* spp. to salinity. This cyclopoid has a wide distribution (e.g. estuaries, open waters, tropical and sub-tropical waters) and shows a high abundance associated with particular physiological characteristics, making *Oithona* one of the most important copepod in food webs (Porri et al., 2007; Turner, 2004). Abundance of zooplankton species is also related to nutrients concentrations and Chl *a* concentration that are higher at the upper estuary, similarly to results of previous studies (Marques et al., 2006, 2007a).

CONCLUSION

The small copepods are important links in marine food webs, serving as major grazers of phytoplankton and as prey for ichthyoplankton and other larger pelagic carnivores (Turner, 2004). The Mondego estuary food chain supports an important fish community (Martinho et al., 2007). Studies performed by Martinho et al. (2008) and Dolbeth et al. (2008), who analyzed the feeding ecology of the main fish species of Mondego estuary, concluded that copepods were an important component of fish diets, mainly for the juveniles of the European sea bass *Dicentrarchus labrax* Linnaeus, 1758. Consequently, high zooplankton densities may lead to important trophodynamic effect in fish community.

The present study documents the role of spatial and temporal variance in the dynamics of small-sized zooplankton fraction in an estuary. The results stressed the species-specific responses in this diverse environment. The challenge for future research lies in disentangling the role of this natural variability and variability due to human impact (e.g. eutrophication, responses to climate change, extreme events).

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CHAPTER II

Response to climatic variability of Copepoda life history stages in a southern European temperate estuary

Chapter II is submitted in the form of a manuscript to Zoological Studies.

ABSTRACT

This study aims to investigate the effects of an extreme climate event (severe drought) on Copepoda ecology. Monthly samples were conducted from 2005 to 2007, at five stations, using a 63 and 335µm mesh nets. Calanoida were represented mainly by *Acartia clausi*, *Temora longicornis* and *Acartia tonsa* and Cyclopoida by *Oithona plumifera* and *Acanthocyclops robusts*. *A. clausi* and *T. longicornis* dominated at the mouth and middle estuary; *A. tonsa* and *A. robustus* were associated to the upper estuary while *O. plumifera* showed the highest densities at the downstream section. Nauplii occurred in higher densities at the mouth. The relationship of copepod assemblages and environmental factors was analyzed using the STATICO method which allowed distinguishing the combination factors that mostly contributed to this relationship. Winter was characterized by high concentrations of nutrients, cold waters and low salinities while summer was related, in general, 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. Copepoda assemblages showed a clear seasonal pattern that superimposed to the inter-annual variability. Moreover, the severe drought was responsible for the predominantly dominance of marine species.

KEYWORDS

Copepods, Life stages, Mondego estuary, Seasonal and inter-annual variability, STATICO.

INTRODUCTION

Estuaries are one of the most productive natural habitats supporting a high number of animals and a wide range of sizes and weights (Mclusky and Elliot, 2004). These ecosystems became interesting areas to study the responses of animals and plants to severe environmental gradients. Estuaries are transition zones between the rivers and the sea differing in biotic (e.g. predation, competition) and abiotic conditions (e.g. temperature, salinity, food quantity and quality). Plankton (and mainly zooplankton) is known to be particularly sensitive to these changes because it is strongly influenced by climatic features and changes in hydrological conditions (Ara, 2001; David et al., 2005; Isari et al., 2007; Marques et al., 2007a).

Several works have focused zooplankton ecology and dynamics (Lam-Hoai et al., 2006; Uriarte and Villate, 2005) namely in the Mondego estuary (Azeiteiro et al., 2000; Gonçalves et al., 2010a, b; Marques et al., 2007a, b, 2009; Primo et al., 2009). Copepods are usually the dominant group of mesozooplankton, playing an important role in the trophic food web since they are a link between producers and secondary consumers (Richmond et al., 2007). Still, information about spatio-temporal distribution of copepods integrating simultaneously nauplii, juveniles and adults life history stages with the aim to examine their ecology over contrasting environmental conditions is scarce in literature, mainly in European southern systems (Kršinić et al., 2007). Studies have been developed to a single species or taxonomic categories (Hansen et al., 2004; Incze and Ainaire, 1994) in a spatio-temporal distribution perspective or performing laboratorial tests to investigate organisms' responses to environmental factors (Cook et al., 2007). Moreover, earlier life history stages (mainly naupliar stages) are pivotal for further development and growth of juveniles and to the maintenance of copepod populations (Cook et al., 2007). Temperature, food quality and quantity are the main environmental factors controlling stage duration of copepods, while preferential salinity regions to lay the eggs are chosen in order to obtain the highest hatch success (Chinnery and Williams, 2004; Cook et al., 2007). Thus, studies not addressing the early copepod stages have no information about changes in species dynamics and thus cannot infer about seasonal fluctuations due to a lack of information. This frequent undersampling of small copepod species, and mainly earlier stages, may lead to a limited view of the ecology of planktonic systems (namely in estuarine systems). Furthermore, in the last years has been raised the interest in climate change impacts in estuarine/marine ecosystems. However, few studies have focused on the impact of large-scale weather events, such as an extreme drought, in Copepoda community, in order to assess a holistic and integrative view of an ecosystem to global climate change. This change associated to biological long time series may contribute to further knowledge about inter-annual variations in abundance and diversity of copepod community.

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

MATERIALS AND METHODS

Study area and sampling sites

The Mondego estuary is a small mesotidal system with 8.6 km^2, located in the western Atlantic coast of Portugal (40º 08´ N, 8º 50´ W) (Fig. 1). It comprises two channels, north and south, separated by the Murraceira island about 7 km from the shore joining again near the mouth. These two arms present different hydrological characteristics. The north arm is deeper (4-8m during high tide, tidal range about 2-3 m), presents a low residence time (<1day) and is the location of the commercial harbour and the main navigation channel. At neap tides, this arm is characterized by a salt-wedge during low tide, changing to partially mixed water column at high tide. At spring tides, it is characterized by a partially mixed water column at low tide and well mixed at high tide (Cunha and Dinis, 2002). The south arm is shallower (2-4m deep, during high tide), has higher residence times (2-8 days) and the water circulation is mostly dependent on the tides and on the 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 the Mondego valley rice fields.

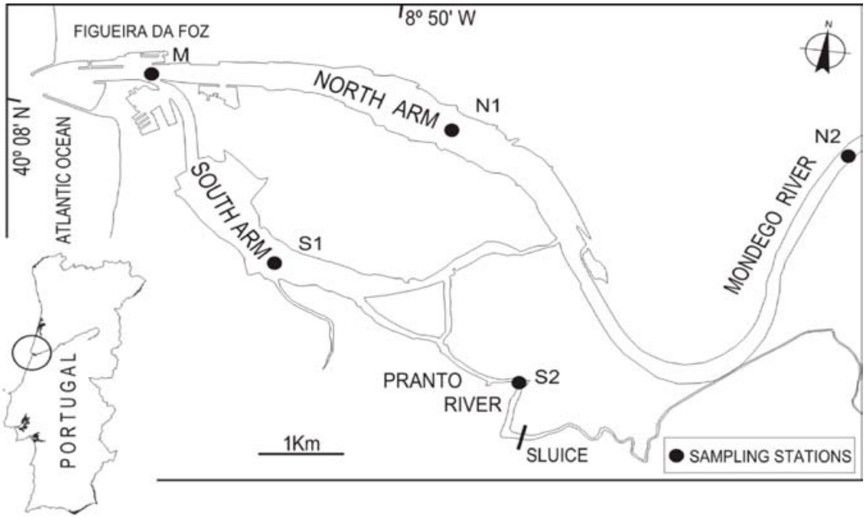

Figure 1 – Location of the Mondego estuary on the western coast of Portugal and the five sampling stations within the estuary: M – mouth station, N1 and N2 – northern arm stations, S1 and S2 – southern arm stations.

Sample collection and laboratorial procedures

Copepod samples were collected monthly in the Mondego estuary from February 2005 to December 2007, during high tide, at five 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 (diameter: 0.5 m) and a 63 µm mesh net (diameter: 0.30 m), equipped with a Hydro-Bios flow meter 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 m^{-3}) and identified to the lowest possible taxonomic level. Water samples were collected on each sampling station to determine nutrient concentrations (Strickland and Parsons, 1972 for nitrates and nitrites, $mg.L^{-1}$; Limnologisk Metodik, 1992 for phosphates and ammonia, $mg.L^{-1}$), Chlorophyll *a* concentration (Parsons et al., 1985 – Chl *a,* $mg.m^{-3}$) and total suspended solids (APHA, 1995 – TSS, $mg.L^{-1}$). Additionally, several hydrological parameters were measured *in situ*: water temperature (ºC) and salinity (WTW Cond 330i), dissolved oxygen concentration (WTW OXI 330i – DO, $mg.L^{-1}$), pH (WTW pH 330i) and transparency with a Secchi disc depth (m). Monthly precipitation and long-term monthly average precipitation (from 1971-2000) were measured at the Soure 13 F/01G station and acquired from INAG – Portuguese Water Institute (http://snirh.inag.pt). Freshwater runoff from Mondego River was obtained from INAG station Açude Ponte Coimbra 12G/01AE, near the city of Coimbra (located 40km upstream).

Data analysis

Only the most abundant *taxa*, having a minimal mean occurrence of 0.1% of the total density observed in the study area were considered. This cut-off eliminated the species that occurred rarely, some being 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 two series of tables (Fig. 2): one for the environmental variables and the other one for species density. Each pair of tables sharing the same sampling period (spring 2005 to autumn 2007) for five sites (M, N1, N2, S1 and S2). Species abundance was transformed using log(x+1) prior to calculations, to minimize the dominant effect of exceptional catches.

Environmental data were normalized by the use of scaling to unit standard deviation within the mode to homogenize the table.

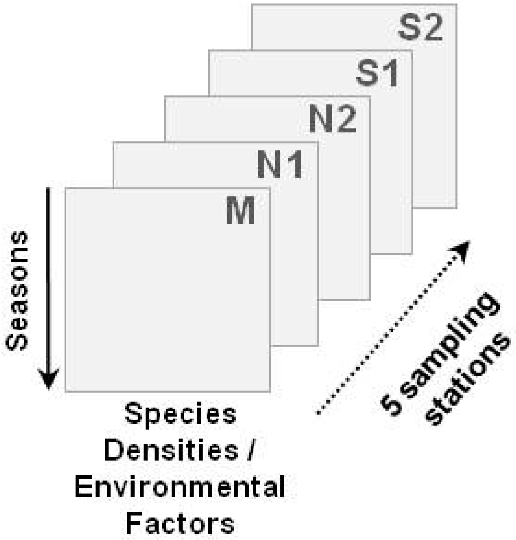

Figure 2 – Three–way data structure: for environmental parameters and species densities at 5 sampling stations.

The common structure between environmental and species density tables and the stability of this structure across the sampling stations were assessed by 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 the spatial and seasonal variability of fish assemblages in Gambia estuary and coastal areas of New Caledonia, respectively, and by Mendes et al. (2009) to describe the spatio-temporal structure of diatom assemblages in Ria de Aveiro (Portugal). This method proceeds in three stages: (1) the first stage consists in analyzing each table by a one-table method (normed PCA of the environmental variables and centered PCA of the species data). The calculation of the vectorial correlations (RV) matrix between stations (in terms of the co-structure between environment and species density) allows the comparison of the stations and the representation of the proximity between stations. The function of this step is to attribute a weight to each station sub-matrix; (2) each pair of tables is linked by the Co-inertia analysis (Dolédec and Chessel, 1994) which provides an average image of the co-structure (species-variables); (3) Partial Triadic Analysis (Thioulouse and Chessel, 1987) is finally used to analyze this sequence. It is a three-step procedure, namely the interstructure, the compromise and the intrastructure (or trajectories) analyses. STATICO also enables to plot the projection of the sampling seasons of each original table on the compromise axes (of the PCA factor map), in terms of species abundances and environmental factors structures. Hence, it is possible to discuss the correlation between species distribution and environmental factors. Calculations and graphs were done using ADE-4 software (Thioulouse et al., 1997). This software is available free of charge at the following Internet address: http://pbil.univ-lyon1.fr/ADE-4.

RESULTS

Climate – precipitation and environmental background

In the Mondego estuary a clear seasonal and yearly variation of rainfall and freshwater discharges was observed during the three-year period (Fig. 3A). In 2005 an extreme drought was recorded with precipitation and freshwater discharge values much lower than the 1971-2000 average, causing one of the biggest droughts of the 20th century in Portugal. A severe reduction was evidenced in freshwater flow with the lowest value in 2005 almost 48-fold lower the highest in 2006. In 2006 and 2007 precipitation values were closer to average except in October 2006 where was registered an above-mean precipitation (Fig. 3A). So, the last two years within 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 almost 14-fold lower than the highest in 2005 (corresponding to the extreme drought). As expected, the highest values of salinity were observed during 2005. Water temperature showed a typical pattern for temperate regions, ranging from 10.12 ± 0.51 ºC to 20.86 ± 2.55 ºC (Fig. 3B).

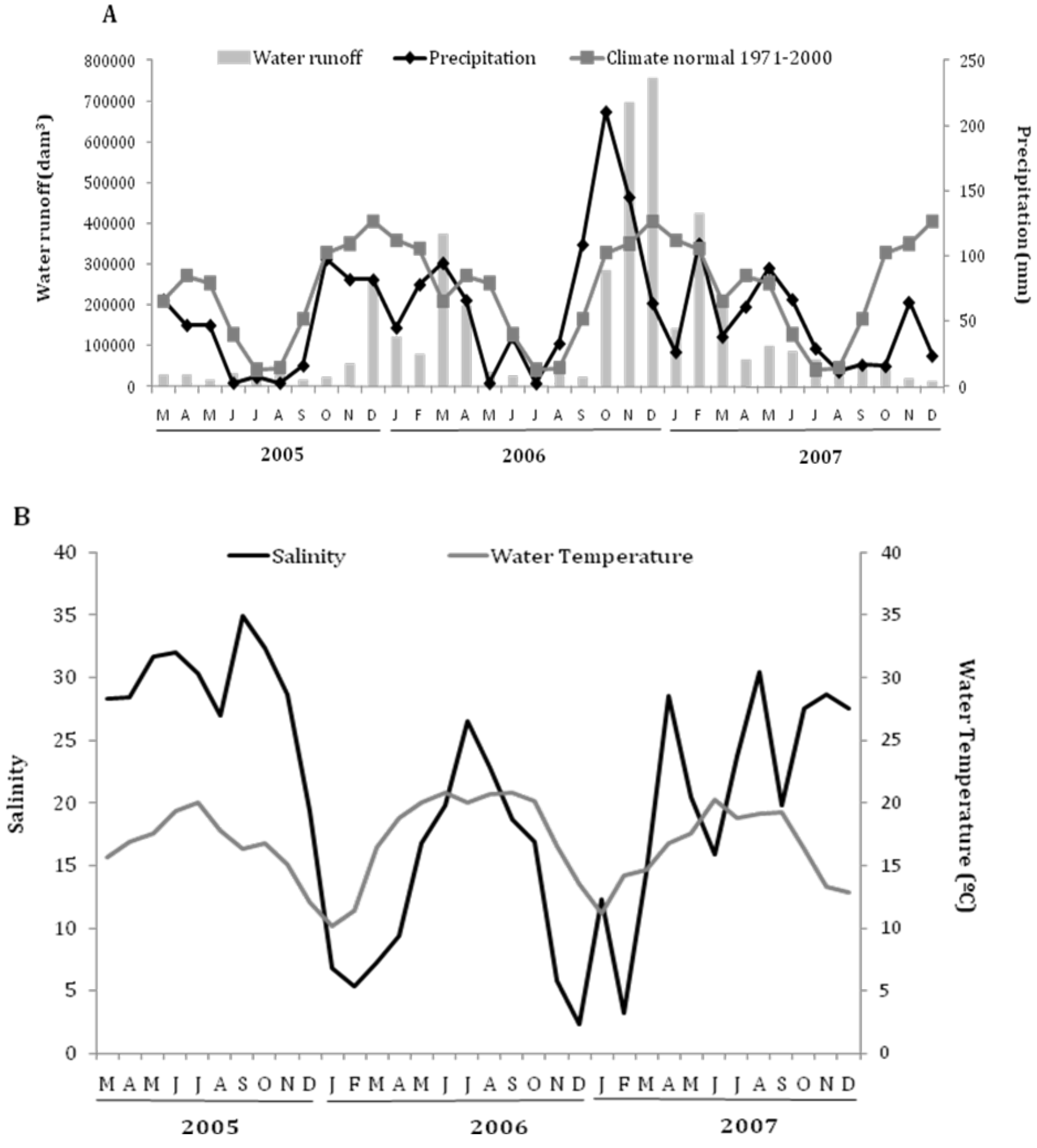

Figure 3 – (A) Monthly precipitation (mm) in Mondego estuary during the study period (from 2005 to 2007). Grey filled squares represent monthly average of 1971 to 2000 (http://snirh.inag.pt) and (B) monthly average of salinity and water temperature (ºC) during the study period (2005-2006 and 2006-2007).

Analysis of the interstructure (between-stations analysis)

From the 55 different Copepoda species identified 28 (including nauplii, copepodites and adults stages) were dominant and occurred regularly. The interstructure factor map of the STATICO analysis, based on the 12 environmental variables (pH, DO, temperature, salinity, transparency, Chl a, TSS and nutrients) and on the 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 decrease order of importance in the compromise, by M, S1, N2 and S2. This means that the compromise will be more influenced by N1, M and S1) (Fig. 4A). The remaining sampling sites (N2 and S2) presented short arrows, meaning that the corresponding tables are less structured being lower its importance in the compromise. The first two axes represented, respectively, 69% and 11% of the total variability (Fig. 4B).

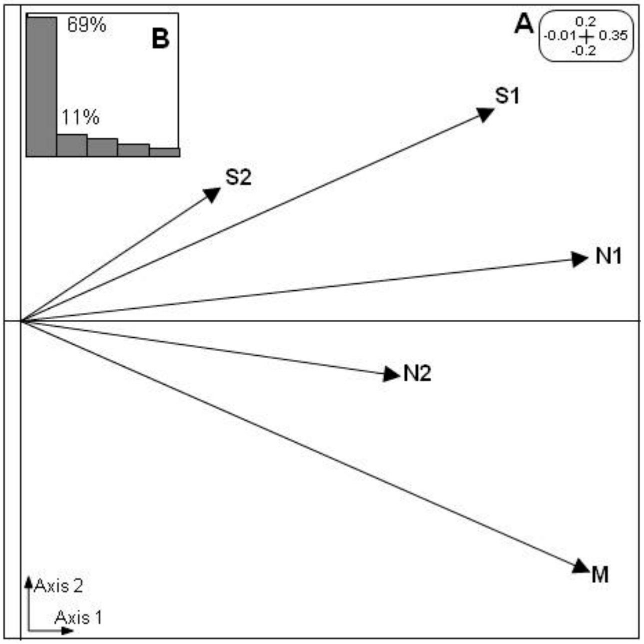

Figure 4 – Interstructure factor map of the STATICO analysis on the Mondego estuary data. (A) This map shows the importance of each sampling station in the compromise (M – mouth station; N1 and N2 – stations of the north arm; S1 and S2 – stations of the south arm). The scales are given in the boxes.

The matrix presenting the RV between the stations sub-matrices (Table 1) showed the strongest correlation (RV = 0.77) observed between the stations S1 and N1 whereas the stations S2 and M pointed out the weakest one (RV = 0.32). From the analysis of the interstructure is possible to analyze the contribution of each sub-matrix in the construction of the compromise (Table 1). They represent the weight of each sub-matrix in the definition of the compromise. It seems that the sub-matrices M, N1 and S1 contributed a larger part in the definition of the compromise suggesting that the other stations had more particular structures leading to a weaker weight. Regarding to $\cos^2$ (Table 1), an indicator of how much the compromise expresses the information contained in each table, the station N1 was the one that fits best ($\cos^2 = 0.81$), followed by stations M and S1 ($\cos^2 = 0.71$ and 0.64, respectively). Lastly, the seasonal dynamics at stations N2 and S2 shows the least accuracy with the compromise ($\cos^2 = 0.54$ and 0.24, respectively), in terms of the co-structure between environment and species density.

Table 1 – Typological value indices: RV = Correlation matrix: contains the cosines between tables; Weights = weights of tables in the compromise; Cos^2 = Square cosine between table and approximated compromise

Sampling sites	**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

Spatial structure

The factor plots of the first two axes of the compromise analysis are shown for the copepod community and the environmental variables (Fig. 5). The first axis was clearly dominant, and accounted for 89% of the explained variance in contrast with the second axis which accounted for 4% of the explained variance being much less significant (Fig. 5C).

Therefore, they provided a good summary and typology of the spatial species organization, on the basis of the common structure, for the sampling sites across the 3 years.

The factor map of the compromise for the copepod community in the STATICO analysis indicates that the most abundant species in the samples were mainly associated with the same environmental variables: temperature and salinity (Figs. 5A and 5B). 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, nitrites, nitrates and silica were low (Figs. 5A and 5B). In particular copepodites of *O. plumifera, E. acutifrons* and copepodites of *E. acutifrons* were clearly influenced by high values of temperature, presenting during the study period abundances of $2.62\times10^4 \pm 1.33\times10^5$ ind.m^{-3}; $3.92\times10^3 \pm 1.44\times10^4$ ind.m^{-3} and $9.59\times10^3 \pm 4.99\times10^4$ ind.m^{-3}, correspondingly. Beyond that, the high density of copepodites of *O. plumifera* ($2.62\times10^4 \pm 1.33\times10^5$ ind.m^{-3}) was also under the influence of high values of phosphates and low concentrations of dissolved oxygen. In opposition were copepodites and adults *of Paronychocamptus nanus* Sars, 1980 ($1.10\times10^3 \pm 5.75\times10^3$ ind.m^{-3}; $3.59\times10^2 \pm 1.52\times10^3$ ind.m^{-3} respectively), copepodites and adults of *Acanthocyclops robustus* G. O. Sars, 1863 ($1.67\times10^3 \pm 1.12\times10^4$ ind.m^{-3}; 1.20 ± 3.76 ind.m^{-3}, respectively) and copepodites of *Centropages* sp. ($6.46\times10^2 \pm 5.48\times10^3$ ind.m^{-3}). These were more abundant in waters of lower salinity and temperature combined with the reduction of transparency and TSS values. Moreover, copepodites of *Acartia* sp. ($1.94\times10^3 \pm 7.97\times10^3$ ind.m^{-3}), copepodites of *Clausocalanus* sp. ($2.19\times10^2 \pm 1.17\times10^3$ ind.m^{-3}), copepodites of *Acartia clausi* Giesbrecht, 1889 ($9.94\times10^2 \pm 3.66\times10^3$ ind.m^{-3}), copepodites of *T. longicornis* ($1.05\times10^3 \pm 5.09\times10^3$ ind.m^{-3}), copepodites of *Oithona* sp. ($1.46\times10^4 \pm 5.00\times10^4$ ind.m^{-3}) and copepodites of *Paracalanus*-type form (calanoid) ($2.22 \times10^3 \pm 8.85\times10^3$ ind.m^{-3}) were abundant when salinity and TSS concentrations were elevated and nitrites, ammonium, pH and phosphates were low (Figs. 5A and 5B). Copepodites of *Pseudocalanus* sp., *Paracalanus* sp. and *Clausocalanus* sp. are referred as *Paracalanus*-type form when they do not show the main characteristics of each genus to their identification. Regardless of species, the first axis of the compromise factor map for environmental parameters mainly described a negative association between salinity and nitrates in addition to silica (Fig. 5B). On this factorial plan pH, ammonium and chlorophyll *a* concentration presented a weak representation (Fig. 5B). The second axis opposed phosphates, as well as temperature and dissolved oxygen.

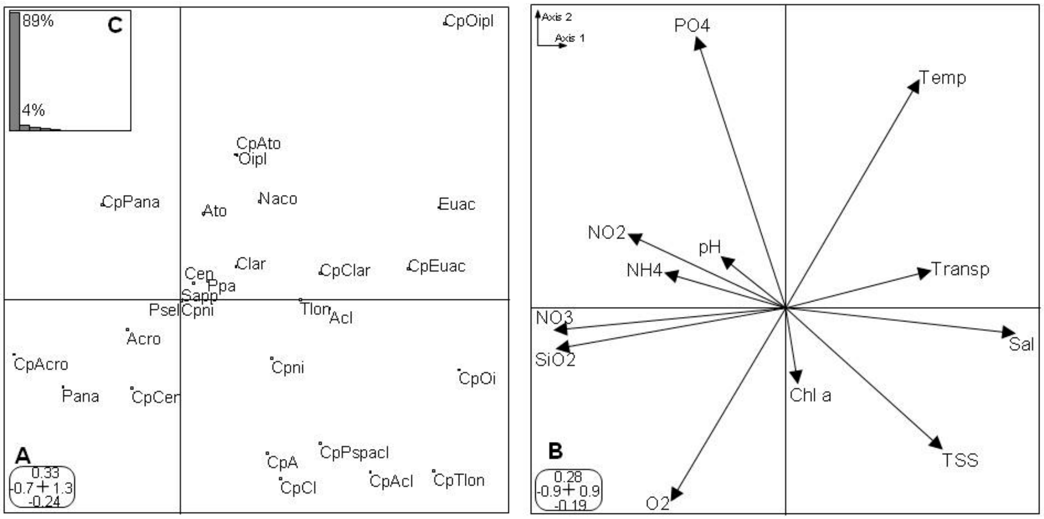

Figure 5 - Compromise factor map of the STATICO analysis of the copepod species variables (A) and environmental parameters (B). (C) Eigenvalues diagram. The scales for axes are given in the boxes. Chl *a* – chlorophyll *a*, Temp – water temperature, Sal – salinity, O2- dissolved oxygen, TSS – total suspended solids, Transp – transparency, PO4 – phosphates, NO2 – nitrites, NO3 – nitrates, SiO2 – silica, NH4 – ammonia. See table 2 for species codes.

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

For each survey, the projection on the compromise axes of the 28 species (see Table 2 for species codes) and of the 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 the sampling stations M, N1 and S1 surveys (Figs. 6A and 6B). The N2 and S2 surveys, also showed similar oppositions, however with a slight difference in the density of copepodites of *A. clausi*, *T. longicornis*, *Acartia* sp., *Clausocalanus* sp. and *Paracalanus*-type form combined with the dynamics of environment over these stations (Figs. 6A and 6B). Species' patterns distribution are more similar among downstream stations, with a high correlation with species abundances and the environment. In upstream stations species presented lower abundances and lower correlation with the environment, mainly in the southern station (S2). At a spatial scale, salinity and temperature were unrelated at downstream stations, whereas at upstream stations these two factors presented a positive correlation (Fig. 6B). Furthermore, nutrients concentration appeared positively correlated on the left side of axis 1, along which the freshwater species *A. robustus* appeared to distribute. Marine species are mostly located on the right-hand side of the first axis, which means higher temperature, salinity and TSS concentration. Moreover, looking at some environmental characteristics, it can be confirmed that the stations M, N1, S1 are mutually more similar, as compared to the other ones.

Table 2 – List of species (including life stages) and their abbreviations used in multivariate analysis (STATICO). Average of copepod densities during the study period is recorded

Taxa		**Abbreviatiaon**	**Habitat**	**Average**
	Nauplii Copepoda	Naco		3.57E+05 (0-5.61E+07)
	Copepodite not identified	Cpni		2.60E+03 (0-4.09E+05)
Calanoida				
Acartiidae	Copepodite *Acartia* sp.	CpA		1.94E+03 (0-3.04E+05)
	Copepodite *Acartia clausi*	CpAcl	Marine	9.94E+02 (0-1.56E+05)
	Acartia clausi	Acl	Marine	8.45E+01 (0-1.33E+04)
	Copepodite *Acartia tonsa*	CpAto	Estuarine	4.30E+03 (0-6.74E+05
	Acartia tonsa	Ato	Estuarine	1.93E+02 (0-3.02E+04)
Temoridae	Copepodite *Temora longicornis*	CpTelo	Marine	1.05E+03 (0-1.64E+05)
	Temora longicornis	Telo	Marine	7.84E+01 (0-1.23E+04)
Centropagidae	Copepodite *Centropages* sp.	CpCen		6.46E+02 (0-1.01E+05)
	Centropages sp.	Cen		9.58E-01 (0-1.50E+02)
Clausocalanidae	Copepodite *Clausocalanus* sp.	CpCl		2.19E+02 (0-3.43E+04)
	Copepodite *Clausocalanus arcuicornis*	CpClar	Marine	5.65E+02 (0-8.88E+04)
	Clausocalanus arcuicornis	Clar	Marine	7.68E+00 (0-1.21E+03)
	Pseudocalanus elongatus	Psel	Marine	3.11E-02 (0-4.88E+00)
Paracalanidae	*Paracalanus parvus*	Ppa	Marine	6.64E+01 (0-1.04E+04)
	Copepodite of *Paracalanus*-type form	CpPspacl		2.22E+03 (0-3.49E+05)
Cyclopoida				

Oithonidae	Copepodite *Oithona* sp.	CpOi		1.46E+04 (0-2.30E+06)
	Copepodite *Oithona plumifera*	CpOipl	Marine	2.62E+04 (0-4.12E+06)
	Oithona plumifera	Oipl	Marine	4.10E+02 (0-6.44E+04)
Cyclopidae	Copepodite *Acanthocyclops robustus*	CpAcro	Freshwater	1.67E+03 (0-2.62E+05)
	Acanthocyclops robustus	Acro	Freshwater	1.20E+00 (0-1.89E+02)
Harpacticoida				
Euterpinidae	Copepodite *Euterpina acutifrons*	CpEuac	Estuarine	9.59E+03 (0-1.51E+06)
	Euterpina acutifrons	Euac	Estuarine	3.92E+03 (0-6.16E+05)
Laophontidae	Copepodite *Paronychocamptus nanus*	CpPana	Marinee	1.10E+03 (0-1.73E+05)
	Paronychocamptus nanus	Pana	Marine	3.59E+02 (0-5.63E+04)
Poecilostomatoida				
Sapphirinidae	*Sapphirina* sp.	Sapp		9.10E-03 (0-1.43E+00)

The major groups of copepods, calanoida, cyclopoida and harpacticoida are represented in the study area. Calanoida were represented mainly by marine species (e.g. *A. clausi* and *T. longicornis*) and by the estuarine species *Acartia tonsa* Dana, 1849. Cyclopoida were mainly represented by euryhaline species *O. plumifera* and the freshwater species *A. robustus. A. clausi* and *T. longicornis* dominated principally at the mouth (M) and middle estuary (N1 and S1) while *A. tonsa* and *A. robustus* were more associated to the upper estuary (N2 and S2). The cyclopoid *O. plumifera*, mostly copepodite stages, occur at along the estuary, showing the highest densities at the mouth (M) and middle north arm (N1). This species (mainly juvenile stages) represents one of the most abundant copepod species. Harpacticoids species are represented by the estuarine species *E. acutifrons* and *P. nanus,* presenting always lower densities than the Euterpinidae species. *E. acutifrons* occurs along the whole salinity gradient, showing a widely distribution in the estuary. Although *P. nanus* is also found at the five sampling stations, this species shows higher densities at middle estuary (N1 and S1). The order Poecilostomatoida is represented by *Sapphirina* sp. This taxon shows the lowest abundance from all the copepods' ($9.10\times10^{-3} \pm 4.47\times10^{-2}$ ind.m^{-3}) being considered a rare species. Nauplii of copepods occur along the whole estuary, still were observed at higher densities at the mouth station (St M).

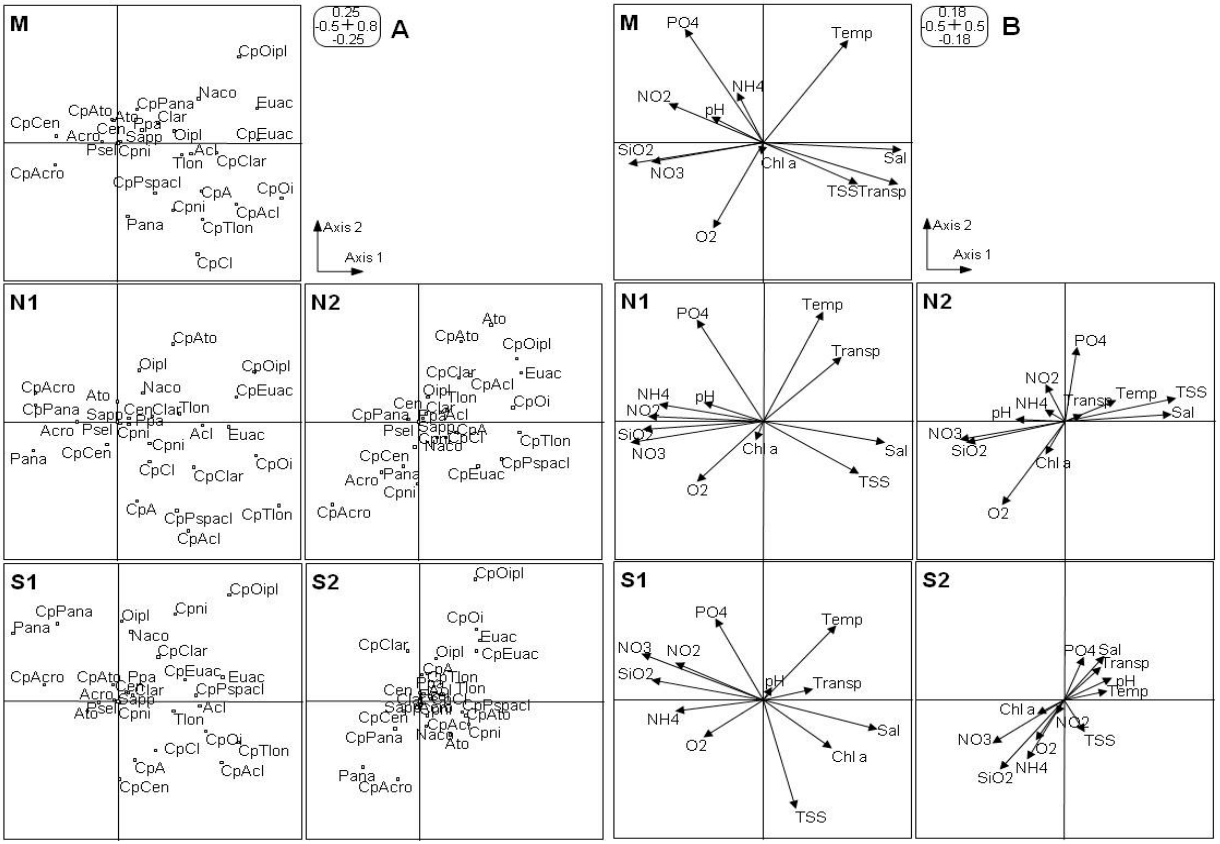

Figure 6 – Trajectories factor maps of the STATICO analysis: (A) projection of the average position of copepod species at each sampling station and (B) projection of the environmental variables at each sampling station (M – mouth station; N1 and N2 – stations of the north arm; S1 and S2 – stations of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

The co-structure graphics (divided according sampling sites) clearly showed the dynamics of species-environment relationships and highlighted differences between seasons (Fig. 7). The projections of the species points (origin of arrows) were located differently according to the site raising their dispersion from the upstream stations (N2 and S2) to the mouth station (St M). These positions were essentially related to the salinity and temperature, with the smallest values observed in upstream stations. Whatever the date, the species points (origin of arrows) were more stable than the environmental points, expressing the steady establishment of the species assemblages in the estuary, in spite of the high environmental variability (principally for salinity, temperature and phosphates). In a general way, winter was mostly projected on the left-hand side of the first axis, characterized by the high concentrations of nitrates, nitrites, silica and ammonium, in opposition to high salinity and warm waters and, for the most part, with high density of juveniles and adults of *A. robustus* and *P. nanus*. On contrary, nauplii of copepods appeared to be numerically less important during winter months. This season was the most regularly projection and only presented exceptions at S1 and S2. Summer season was characterized by the factors that described axis 2, which means that at stations M, N1, S1 and N2 summer was mainly under the influence of high values of phosphates, salinity, temperature, TSS and dissolved oxygen. By other hand, for S2 summer was influenced by highest phosphates, transparency, salinity, pH and temperature. From a species point of view summer was principally characterized by high density 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. The exceptions were at S1 for autumn (mostly projected on the right-hand side of the first axis) and N2 for spring (mostly projected on the left-hand side of the first axis). The arrows were mostly short for summer, expressing a good coincidence between environmental conditions and plankton structure. The exception occurred at N2, with a poor fit between the copepod species' densities and environment (long arrows) for some surveys in summer 2005. However, at this season, there was a higher correlation between the distribution of copepod densities and the environment under the direct influence of high values of phosphates, salinity, TSS, dissolved oxygen and temperature at the downstream stations (M, N1 and S1).

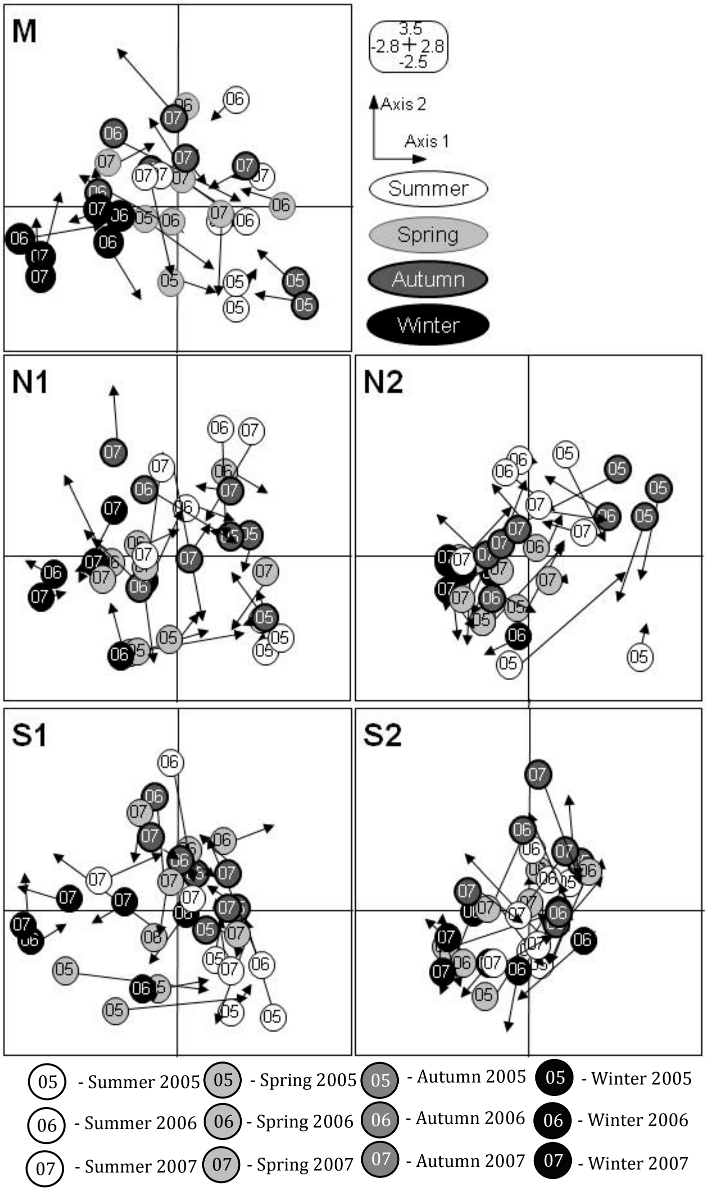

Figure 7 - Trajectories factor plots of the STATICO analysis: projection of the seasons along the study period (2005, 2006 and 2007) in terms of both environmental and copepod structure. Graphs are given for each sampling site (M – mouth station; N1 and N2 – stations of the north arm; S1 and S2 – stations of the south arm) that showed the highest contribution to the co-structure between environmental factors and copepod abundances. Each sample is represented by two points: one is the projection of the row of the species table (circle: origin of arrows), and the other is the projection of the row of the environmental table (end of arrows). The length of the connecting line reveals the disagreement or the consensus between the two profiles (species–environment), i.e., the length of the line is proportional to the divergence between the datasets. When the datasets agree very strongly, the arrows will be short. Likewise, a long arrow demonstrates a locally weak relationship between the environment and copepod features for that case. The scales for axes are given in the boxes.

Furthermore, there is a strong co-structure between species densities and environmental factors at upstream station of the southern arm (S2) under the influence of high values of phosphate and salinity, transparency, temperature and pH, in summer months. By other hand, at S1 all winter surveys presented short arrows expressing a strong co-structure between species and environment, which means that the environmental factors (high values of nitrates, nitrites, silica and ammonia and low values of temperature and salinity concentrations) explained well the distribution of species at this season. At upstream stations (S2 and N2) the arrows were mostly long which means a lower correlation between the distribution of copepod densities and the environment.

DISCUSSION

This study focused the copepod species and respective life stages mostly representative of the Mondego estuary. Moreover, it is also examined the relationship between environmental factors and species densities and distribution, under along three years of study. Indeed, in the Mondego estuary, as in other coastal areas (Kibirige and Perissinoto, 2003; Leandro et al., 2007; Uriarte and Villate, 2005) Copepoda was the main dominant group. 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 characteristic of low values of salinity and high nutrients concentration and dissolved oxygen.

Concerning seasonal variations, winter is marked by a higher presence of freshwater species and lower densities of nauplii. This abundance and distributional patterns were also observed in other works (Gonçalves et al., 2010a, b; Primo et al., 2009; Tackx et al., 2004). In spring/summer months there are 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 the small marine cyclopoids of the genus *Oithona* and *Oncaea,* related with saline waters, at the estuarine systems of the Basque coast. Moreover, *Centropages typicus* Krøyer, 1849, *Oithona nana*

Giesbrecht, 1892 and *P. parvus* are typically surface species with high temperature and low salinity affinities whilst *O. plumifera*, *Clausocalanus* sp., *P. elongatus* and *T. longicornis* were reported as species preferring deeper waters with lower temperature and higher salinity concentration. Additionally, several authors (e.g. Holste and Peck, 2006; Villate et al., 1993) stated that copepod nauplii distribute towards higher salinities, however, due to its high sensitivity to environmental factors, it may change from species to species and from adults to juveniles and nauplii of the same species (Chinnery and Williams, 2004). According to Incze and Ainaire (1994) the peak of occurrence of copepod nauplii is on latter spring related with the greatest concentrations of Chlorophyll *a*. Chinnery and Williams (2004) stated that, differential distribution of different life stages in estuarine systems may be due to the poorly swimming activity of the naupliar stages. The weak ability to swim leads nauplii into areas of higher salinities and swept them towards the mouth of the estuary where they stay and develop to later stages moving afterwards back up the estuary to take up their adult distribution patterns.

At the Mondego estuary, freshwater species show higher densities at upstream stations (N2 and S2) because of the high influence of the freshwater from the Mondego and the Pranto rivers. Marine and estuarine species are found at the middle stations (N1 and S1) due to the intrusion of marine water in both stations, while at the mouth station (M) a higher number of marine species is verified (Gonçalves et al., 2010b; Marques et al., 2007a, b; Primo et al., 2009).

Downstream stations (St M, St S1 and St N1) exhibited a strong correlation, together with the most abundant species mainly associated with salinity and temperature, presenting a rich marine fraction. This fact agrees with other studies carried out in a wide range of European estuaries (e.g. Mouny and Dauvin, 2002; Tackx et al., 2004), that emphasized the significant influence that salinity has on zooplankton abundance, composition and distribution. The higher salinities (and low freshwater inflow) registered in 2005 (the Portuguese Weather Institute - http://web.meteo.pt/clima.jsp - classified the drought period of 2005 as the worst drought of the past 60 years) lead to an increase of marine zooplankters in the estuary. The same was reported by Marques et al. (2007a) and Primo et al. (2009) for the mesozooplankton and by Martinho et al. (2007) for fish species. The severe drought in 2005 was responsible for a clear spatial shift of copepod community predominantly dominated by marine species, which remained along the next regular climatic years (2006 and 2007). Together with the marine spatial preferences of naupliar forms, lead to a clear absence of an inter-annual distributional pattern in the Copepoda assemblages. Seasonality was also detected along the study period, driving to zooplankton assemblage's variability in Mondego estuary. This study also concluded about the naupliar and copepodites stages, and also determined spatial patterns of distribution. *A. tonsa* and *A. clausi* are one of the copepods with higher abundance contributing to the increase of copepod dominance in the Mondego estuary. The former exhibit higher densities at upstream stations (N2 and S2) and the latter appear in much higher densities at downstream stations (M, N1 and S1) (Gonçalves et al., 2010b; Marques et al., 2007a, b; Primo et al., 2009). At the estuaries of Bilbao (polluted estuary) and Urdaibai (undisturbed estuary), located on the Basque coast (Bay of Biscay) with salinities varying between 35 and 31, Uriarte and Villate (2005) stated that responses of copepods may be related with water desalination and their tolerance to pollution. These authors referred *A. clausi* and *P. parvus* as the most abundant species in Bilbao and Urdaibai estuaries. Still, at the latter estuary higher densities of *P. elongatus, E. acutifrons* and harpacticoids were also referred. A significant reduction of copepod species with a decrease of salinity values at the polluted estuary was observed, whilst *E. acutifrons* and *Oithona helgolandica* Claus, 1863 did not showed significantly differences, which is in agreement to their wide spread distribution. Similarly to our results, some authors (e.g. Ara, 2001; Uriarte and Villate, 2005) stated that *E. acutifrons* appeared without a clear seasonal trend, occurring along the year, and tolerate a wide range of salinity and temperature. The species' highest abundance is found in estuaries and coastal waters rather than offshore waters (Björnberg, 1963). Uriarte and Villate (2005) reported a diminishment of *A. clausi*, *P. parvus* and *O. plumifera* with the decrease of salinity, with the former species reaching higher abundances in spring. Besides, the distribution of *A. clausi* is affected by the presence of other species - *Acartia bifilosa* Giesbrecht, 1881, - which does not survive so well at higher salinities as do *A. clausi*. Moreover, *A. clausi* species shows higher hatch success at salinities of 33.3 than *A. bifilosa* (Chinnery and Williams, 2004; Uriarte and Villate, 2005). Indeed, *A. tonsa* seems to have a physiological plasticity in terms of egg hatch being the most tolerant of the *Acartia* congeners, with a great hatch success at a range of salinity concentrations (from 15.5 to 33.3) (Chinnery and Williams, 2004). In terms of distribution, *A. tonsa* is a common species in estuaries and European seas, occurring from temperate to subtropical waters, restricting its distribution to habitats with high levels of food (David 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 the north European estuaries characterized by a peak of abundance in late summer and autumn and second spring peak observed in the southern European estuaries. Two assumptions could explain these peaks of abundance: 1) warmer conditions as temperature is known to be the main factor controlling the biological cycle of copepods (Gaudy, 1972) and the world distribution of *A. tonsa* (Conover, 1956); 2) diapauses eggs hatch when water temperature exceeds 10ºC and population rapidly increase above 15ºC (McAlice, 1981) explaining the spring peak and high levels of abundance in the autumn. Indeed, David et al. (2007) reported that abundances of *A. tonsa* are significantly and negatively correlated with suspended particulate matter concentrations and positively and significantly correlated with water temperature and salinity. *P. elongatus* and p-calanus showed higher peaks of abundance in winter-spring, rising with higher values of salinity, whereas *Oncaea media* and *Temora stylifera* Dana, 1849 occurred at higher densities in summer along the salinity gradient.

In the Adriatic Sea system was regarded inter-annual and seasonal variability for some copepod groups over flooded and warmer events, respectively (Kršinić et al., 2007). Nauplii was the most numerous fraction of all copepods groups, showing the highest abundances during warmer and saltier conditions that were referred by the authors as an atypical distribution which may be related to specific summer currents. Moreover, harpacticoids' highest abundances were also reported at warmer and saltier conditions, with the highest values occurring in summer and autumn months. Calanoids and oithonids highest densities were observed in warmer months, which may be related with 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, affecting the juveniles and growth rate. Moreover, adults may be tolerant to a wide range of salinity, but the earlier stages (nauplii and juveniles) may be not. Thus, the vulnerability of nauplii to environmental conditions, mainly salinity and temperature, can threat the maintenance of its populations as they have a narrow survival limits, suffering from a great amount of mortality (Chinnery and Williams, 2004). Moreover, since copepods are an important component of the diet of bentho-pelagic invertebrates and peaks or absence of some copepod population may influence the presence and life cycle of others populations (Carrasco et al., 2007), their dynamics allows us to infer about ecological changes in aquatic systems. Therefore, more studies must be conducted in terms of populations' distribution (and so life-history stages) to understand the relationships among species and between species development and the environment. Thus, it may give the chance to delineate the life cycle of species, contributing to a further knowledge of populations and speculate about the relationships among them and the contribution of each one in the trophic food web.

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CHAPTER III

Seasonal and diel vertical behavior of Copepoda assemblages (naupliar, copepodites and adults) at the boundary of estuarine and coastal waters

Chapter III is submitted in the form of a manuscript to Estuarine, Coastal and Shelf Science.

ABSTRACT

Seasonal, tidal, lunar and diel vertical migrations accomplished by horizontal movements were examined on the main copepod fraction of the Mondego estuary. Seasonal samples were conducted hourly at the mouth of the estuary, during diel cycles (25h), both over neap and spring tides, at the bottom and surface, using a 63μm and 335μm mesh size nets. Simultaneously, four sampling sites inside the estuary were conducted during flood tide. Spring-spring tide revealed to be the situation that best expresses the stable part of copepod-environment dynamics. *Acartia tonsa* and *Oithona nana* distributed mainly at the bottom during ebb tides. A clear resident estuarine performance was noticeable in *O. nana* proving the estuarine preferences of the species. Neritic species showed preferences by saline waters, whereas the resident species distributed mainly at estuarine areas. Copepodites stages showed a similar distribution pattern as estuarine species, avoiding leaving the estuary. In contrast were nauplii and *O. plumifera* that showed higher densities at surface flood tides. Indeed, vertical migrations accomplished by horizontal movements were mainly influenced by depth and tidal cycles, whereas day and night were not ecologically significant.

KEYWORDS

Copepoda, Life history stages, Vertical migration, Horizontal movements, Temperate estuarine system.

INTRODUCTION

Since several decades diel vertical migration accomplished by horizontal transport in invertebrates and fishes has been recognized. Nevertheless only a few years ago it has been reported over tidal timescales (Forward Jr. and Tankersley, 2001). In estuarine and coastal areas the transport by tidal currents is an efficient method for fast horizontal movements, mainly to earlier stages due to their limited swimming abilities. Even so, it also reduces the energy necessary to those movements in organisms reported as good swimmers (Metcalfe et al., 1990). Moreover, tidal-stream transport is used mainly during the night when predation by visual predators is greatly reduced, and thus losses of individuals (Zeng and Naylor, 1997). Furthermore, a similar mechanism is associated with the larval releasing activity. Ovigerous females release their larvae during the crepuscular phase which is probably an adaptation process to avoid predation by visual feeders (Paula, 1989; Zeng and Naylor, 1997). Indeed, several studies had focused in the synchronism between the rhythmic cycles of decapod larval export and the light and lunar phases, following an export strategy of their life cycle (Gonçalves et al., 2003; Paula, 1989; Queiroga et al., 1994, 1996, 1997). Another less studied factor is the migratory behavior of the different life history stages, only studied yet on few species, mainly fish (e.g. the Atlantic salmon *Salmo salar,* Rommel & McCleave 1973 and the European ell *Anguilla anguilla,* Berg 1979) and crabs (e.g. *Carcinus maenas*, Linnaeus 1758 and *Callinectes sapidus*, M. J. Rathbun, 1896) (Forward Jr. and Tankersley, 2001). Concerning this topic there is a lack of knowledge on Copepods communities that are undoubtedly one of the most abundant zooplankton groups.

In the last decades it has been highlighted the importance of planktonic copepods in the dynamic of aquatic food webs (Damotharan et al., 2010; McLusky and Elliot, 2004). They play a pivotal ecological role in terms of biomass and energy fluxes transference between primary producers and higher trophic levels, being raised the importance of this group in ecological studies (Damotharan et al., 2010; Kršinić et al., 2007).

Several works have been performed in coastal, oceanic and estuarine waters in order to study the distribution and vertical heterogeneity of zooplankton (Hsieh et al., 2010; Marques et al., 2009; Morgado et al., 2003). It is now assumed that the vertical distribution patterns of copepods along the year are a dynamic complex structured response to tidal, diel and lunar cycles and to a gradient of environmental variables (e.g. temperature, salinity, hydrostatic pressure), as well as to reproductive cycles of predators (e.g. fish) and prey (e.g. phytoplankton) (Forward Jr. and Tankersley, 2001).

Still, studies about seasonal responses of copepod communities, and respective life history stages, to changes of environmental factors linked with tidal, lunar and diel cycles at different depths are scarce or null in southern European latitudes, being this the first study conducted in the Mondego estuary on this small-sized zooplankton fraction. In fact, naupliar stages make up the numerical bulk of copepods, whilst smaller copepods normally dominate in terms of abundance, biomass and productivity. However, these small fractions are significantly under-represented in many current and historical data sets, limiting the knowledge of copepod populations (Gallienne and Robins, 2001; Titelman and Fiksen, 2004).

The main aim of this work is to study the most dominant marine copepod fraction and the resident estuarine species, and respective life history stages (nauplii, copepodites and adults), in order to: 1) understand the correlation between copepod distribution and the physical properties of water column over seasonal, tidal, lunar and nycthemeral dynamics; 2) to evaluate diel vertical migration and horizontal movements in the distribution of each species and respective life stage at the mouth of the estuary; 3) to compare and evaluate the distribution of copepod species in inner stations and at the mouth of the Mondego estuary. In this paper a first characterization of life cycles of the main copepod species was attempted in order to relate the distribution of the different life history stages of each species.

MATERIALS AND METHODS

Study area

The Mondego River estuary is a small mesotidal system located in the western Atlantic coast of Portugal (40º 08´ N, 8º 50´ W) (Fig. 1). The hydrological basin of the Mondego, with an area of 6 670km^2, provides an average freshwater flow rate of 8.5×10^9 m^3 s^{-1}. Its terminal part is divided into two arms (north and south), separated by Murraceira Island at about 7 km, that converge again near the mouth. Here the influence of both river flow and neritic waters is strong and the depth is around 6-13m. Tides in this system are semi-diurnal, and at the inlet the tidal range is 0.35-3.3m.

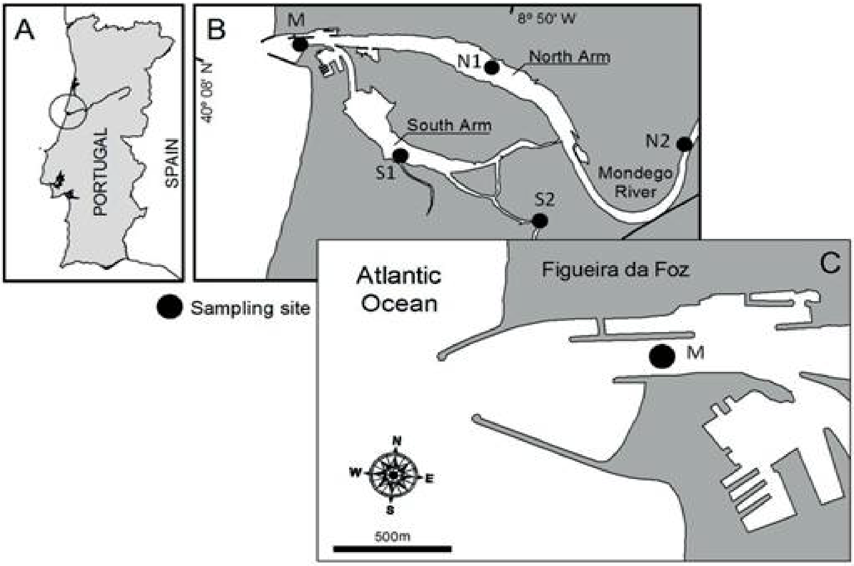


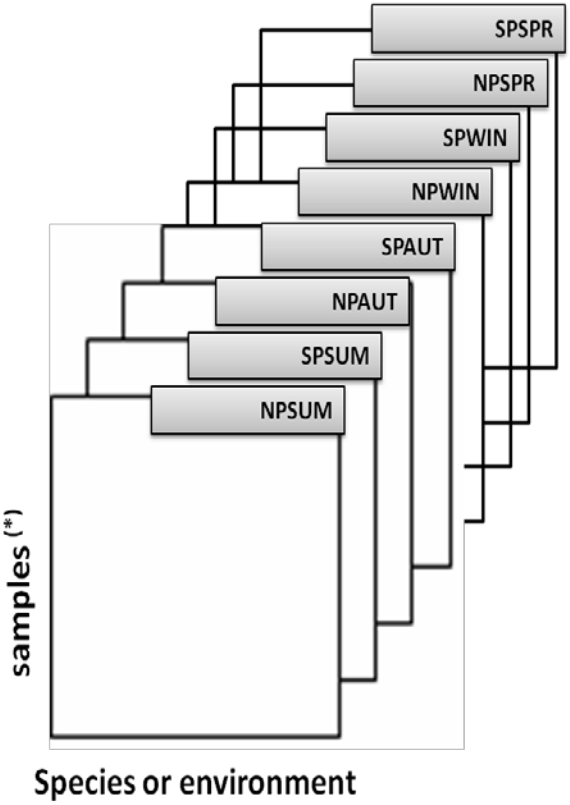
Figure 1 - Location of the Mondego estuary on the western coast of Portugal and the five sampling stations within the estuary: M – mouth station (sampling over diel cycles – 25h); N1 and N2 – northern arm stations; S1 and S2 – southern arm stations.

Field sampling and laboratory analysis

Zooplankton samples were collected at a fixed station located at the mouth of the Mondego estuary (Fig. 1). Samples were collected hourly, from two depths ranges – sub-surface and near the bottom (6-13m), - over diel cycles (25h). Samples were classified as day or night, where the day phase was taken from sunrise to sunset, and the night phase, the complementary period. Seasonal sampling took place in June 2005 (summer), September and October 2005 (autumn); December 2005 (winter) and March and April 2006 (spring), over neap and spring tides. Simultaneously, monthly spatial sampling was performed during flood tide inside the estuary at four stations: N1 and N2 in the north arm; S1 and S2 in the south arm (Fig. 1). The samples were collected with a 335 µm mesh Bongo net (diameter: 0.5 m) and a 63 µm mesh net (diameter: 0.30 m), equipped with a Hydro-Bios flow meter in the mouth (to estimate the volume of water filtered by the nets). Samples were fixed and preserved in 4% buffered formaldehyde in seawater and returned to the laboratory for analysis. The contents of each sample were examined and identified to the lowest possible taxonomic level under a stereoscopic microscope. The samples with a very high numbers of zooplankton organisms were sub-sampled using a Folsom plankton splitter. On all samples and a minimum of 500 individuals were counted, with densities calculated and expressed as individuals/m^3. In parallel to each sampling event, several hydrological parameters were measured *in situ*: water temperature (ºC), salinity (S), dissolved oxygen concentration (DO, mg.L^{-1}) and pH were recorded with appropriate sensors (WTW) at both depths; transparency was measured with a Secchi disc depth (m). Additionally, water samples were collected on each sampling station to determine nutrient concentrations (Strickland and Parsons, 1972 for nitrates and nitrites, mg.L^{-1}; Limnologisk Metodik, 1992 for phosphates and ammonia, mg.L^{-1}). Chlorophyll *a* concentration (Chl *a*) and suspended particulate matter (SPM) were also determined by filtering 500-1000 mL of water sample.

Statistical analysis

From a total of 115 species of zooplankton identified belonging to 18 taxonomic groups, were selected the 5 most regular and abundant copepod species and stages (4 neritic species and a resident estuarine species), plus nauplii of copepod and other copepodites (belong to other copepod species identified), to investigate diel vertical distribution throughout the seasonal cycle. Copepods species' density and environmental factors for each season, lunar phase and tide were combined to generate two series of tables (Fig. 2): one for 12 environmental parameters and the other for 5 species densities (including nauplii, juveniles and adults), plus nauplii and other copepodites (the most common species were selected in order to decrease the number of zero in the analyses). Each pair of tables share the same sampling occurrence (in rows) for 4 seasons (spring, summer, autumn and winter), 2 lunar conditions (neap and spring), 2 depths (sub-surface and bottom), the diel cycle (daylight and dark period) and 2 tidal conditions (ebb and flood). Therefore, each series of tables (biological and environmental) was composed of 16 matrices. Prior to calculations species density was log(x + 1) transformed, to minimize the dominant effect of exceptional values and environmental data were normalized.



NPSUM – Neap-tide Summer; NPWIN – Neap-tide Winter.
SPSUM – Spring-tide Summer; SPWIN – Spring-tide Winter.

NPAUT – Neap-tide Autumn; NPSPR – Neap-tide Spring.
SPAUT – Spring-tide Autumn; SPSPR – Spring-tide Spring.

Figure 2 – Three-way data structure: for environmental parameters and species densities at the mouth of the estuary.

The STATICO method (Simier et al., 1999; Thioulouse et al., 2004) was carried out to analyze the two series of tables coupled two by two. In this study the common structure between environmental and species density tables and the stability of this structure over the sampling period were assessed. The samples, which must be the same for both paired tables, but may vary between the pairs, correspond to a diel cycle reflecting the semi-diurnal tidal cycle. The STATICO method proceeds in three stages: (1) the first stage consists in analyzing each table by a one-table method (normed PCA of the environmental variables and centered PCA of the species data); (2) after that, each pair of tables is linked by the Co-inertia analysis (Dolédec and Chessel, 1994) which provides an average image of the co-structure (species-variables cross-table); (3) Partial Triadic Analysis (Thioulouse and Chessel, 1987) is finally used to analyze this sequence. It is a three step procedure, namely the interstructure, the compromise and the intrastructure (or trajectories) analyses. STATICO also enables to plot the projection of the sampling data in respect to the depth, diel and tidal cycle, of each original table on the compromise axes, in terms of species density and environmental factors structures. Hence, it is possible to discuss the correlation between species distribution and environmental factors. Calculations and graphs shown in this work were done using ADE-4 software (Thioulouse et al., 1997). This software is available at http://pbil.univ-lyon1.fr/ADE-4.

RESULTS

Environmental factors

In table 1 the hydrological conditions recorded during the study period are listed. The water temperature varied between 20.77±1.47ºC, in summer (neap-surface), and 10.75±0.70 ºC, in winter (spring-surface). In general, the higher values of salinity (close to 35) were recorded at all seasons at the bottom at both lunar phases, with exception in summer (Table 1). The lowest values were registered in winter, at the surface (4.96±1.72, neap tide; 9.50±4.61, spring tide). Considering Chl *a,* the concentrations were higher in summer (7.05±6.21 mg.L^{-1}) and winter (6.85±2.06 mg.L^{-1}), showing slightly differences between lunar and tidal cycle. Concerning nutrients concentrations, phosphates and nitrites showed the lowest values, followed by ammonia, nitrates and silica. Mean silica values were generally higher at the surface at both lunar phases. Suspended particulate matter (SPM) and particulate organic matter (POM) showed slightly seasonal differences, with lower values in winter and spring. pH varied from 6.79 ± 0.98 (in winter at spring tide) to 8.25 ± 0.59 (in spring at spring tide), with the lowest value in winter. Considering dissolved oxygen (DO), values ranged from 7.96 ± 0.32 $mg.L^{-1}$ (in autumn) and 10.65 ± 0.52 $mg.L^{-1}$ (in winter).

Copepod assemblages' distribution and dynamics over seasonal, lunar, tidal and day-night cycles

Copepod assemblages were dominated by the marine cyclopoid species (*Oithona plumifera* Baird, 1843), the estuarine cyclopoid species (*Oithona nana* Giesbrecht, 1892), the marine calanoid species (*Acartia clausi* Giesbrecht, 1889 and *Temora longicornis* O. F. Müller, 1785), the estuarine calanoid species (*Acartia tonsa* Dana, 1849) and copepod nauplii. A high number of copepodites from other species were also present.

All species showed the highest densities in the bottom and almost all, with exception to *A. tonsa* (adults and juveniles) and *O. nana* (adults and juveniles), occurred mainly in neap tide (Fig. 3). Moreover, at this lunar phase, nauplii of copepods, *A. clausi* (juveniles and adults), copepodites of *T. longicornis* and other copepodites showed higher densities in summer at flood tide during night (FN) (Figs. 3A, 3C, 3E, 3K and 3W). In winter and spring, at both lunar phases, these species showed lower densities or did not occur (Figs 3A-3F, 3K, 3L, 3W and 3X). *O. plumifera* (adults and juveniles) and *T. longicornis* showed their peaks of abundance in autumn (neap tide), at ebb tide, with the adults of *O. plumifera* occurring mainly during the day (ED), while the juveniles of *O. plumifera* and *T. longicornis* appeared at higher densities in the night (Figs. 3G, 3I and 3M). Considering the estuarine species *A. tonsa* (adults and juveniles) and *O. nana* (adults and juveniles) their occurrence in neap tide was very low or null, showing the highest densities in spring tide (Figs.3O-3V). Adults of *A. tonsa* only occurred in summer, with their peak of density at flood tide during night (FN) while the juveniles were observed in winter mainly at ebb tide (ED and EN) (Figs. 3T and 3V). *O. nana* (adults and juveniles) were presented in autumn (mainly the adults) and winter showing higher densities in winter at ebb tide during the night (EN) (Figs. 3P and 3R).

Table 1 – Environmental parameters (mean ± SD) measured during the studied period, at spring-neap tide and at surface-bottom deepness.

Season	Lunar phase	Depth	NH_4 (mg.L^{-1})	PO_4 (mg.L^{-1})	Si (mg.L^{-1})	NO_2 (mg.L^{-1})	NO_3 (mg.L^{-1})	Chl *a* (mg.L^{-1})	pH	O_2 (mg.L^{-1})	Temp (ºC)	Sal (‰)	SPM (g.L^{-1})	POM (g.L^{-1})
Summer	**Neap**	**S**	0.03±0.02	0.01±0.01	0.27±0.13	0.00±0.00	0.04±0.03	7.05±6.21	8.10±0.18	9.47±0.70	20.77±1.47	29.87±3.05	0.05±0.03	0.01±0.01
		B	0.04±0.02	0.01±0.00	0.14±0.07	0.00±0.00	0.02±0.03	4.25±2.75	8.03±0.19	9.60±1.03	18.64±0.80	33.37±0.61	0.07±0.05	0.01±0.01
	Spring	**S**	0.05±0.02	0.03±0.01	0.24±0.12	0.01±0.01	0.03±0.03	3.59±1.77	8.14±0.08	9.14±0.78	18.99±0.85	26.08±7.12	0.03±0.01	0.01±0.00
		B	0.05±0.02	0.03±0.01	0.24±0.14	0.01±0.00	0.03±0.03	3.99±1.03	8.18±0.07	9.12±0.48	18.28±0.65	26.58±8.08	0.04±0.01	0.01±0.00
Autumn	**Neap**	**S**	0.06±0.02	0.05±0.01	0.61±0.18	0.04±0.01	0.22±0.09	2.50±1.52	7.72±0.32	7.96±0.32	17.85±0.94	28.81±3.24	0.03±0.01	0.01±0.00
		B	0.03±0.01	0.03±0.01	0.28±0.07	0.03±0.01	0.08±0.03	2.84±0.99	7.84±0.27	8.77±0.96	15.98±0.39	35.19±0.64	0.03±0.01	0.01±0.00
	Spring	**S**	0.03±0.02	0.03±0.01	0.44±0.18	0.00±0.00	0.26±0.15	3.79±2.40	7.95±0.40	9.12±0.84	15.71±0.74	32.05±4.06	0.03±0.01	0.01±0.00
		B	0.02±0.02	0.03±0.01	0.30±0.11	0.00±0.00	0.16±0.08	5.56±4.13	7.86±0.23	9.65±0.70	15.35±0.37	34.64±1.93	0.03±0.00	0.01±0.00
Winter	**Neap**	**S**	0.10±0.04	0.03±0.01	1.63±0.55	0.01±0.00	0.93±0.23	6.85±2.06	7.38±0.38	9.81±0.53	11.97±0.37	4.96±1.72	0.01±0.00	0.00±0.00
		B	0.04±0.02	0.01±0.01	0.42±0.32	0.01±0.00	0.21±0.17	3.68±1.40	7.37±0.43	9.04±0.82	13.64±0.52	31.62±6.33	0.03±0.01	0.00±0.00
	Spring	**S**	0.11±0.06	0.02±0.01	1.53±0.20	0.01±0.00	0.89±0.22	4.62±2.07	6.79±0.98	10.65±0.52	10.75±0.70	9.50±4.61	0.01±0.00	0.00±0.00
		B	0.04±0.01	0.01±0.01	0.33±0.13	0.01±0.00	0.22±0.07	3.79±3.75	6.86±0.59	10.08±0.47	12.90±0.39	34.19±1.61	0.03±0.00	0.00±0.00
Spring	**Neap**	**S**	0.91±0.24	0.04±0.01	2.13±0.43	0.02±0.01	1.25±0.31	3.54±0.73	7.38±0.35	9.96±0.76	12.43±0.41	20.93±13.26	0.01±0.01	0.00±0.00
		B	0.07±0.08	0.02±0.01	0.53±0.16	0.01±0.00	0.37±0.14	3.15±1.26	7.49±0.26	9.34±0.40	12.38±0.74	17.20±13.37	0.04±0.02	0.01±0.00
	Spring	**S**	0.08±0.03	0.03±0.00	1.62±0.42	0.01±0.00	0.69±0.27	2.63±1.11	8.25±0.59	8.64±0.58	15.38±1.64	10.35±5.20	0.01±0.00	0.00±0.00
		B	0.02±0.02	0.01±0.01	0.64±0.34	0.01±0.01	0.31±0.17	1.97±0.64	8.21±0.29	9.21±0.91	15.34±0.65	29.15±4.82	0.02±0.00	0.00±0.00

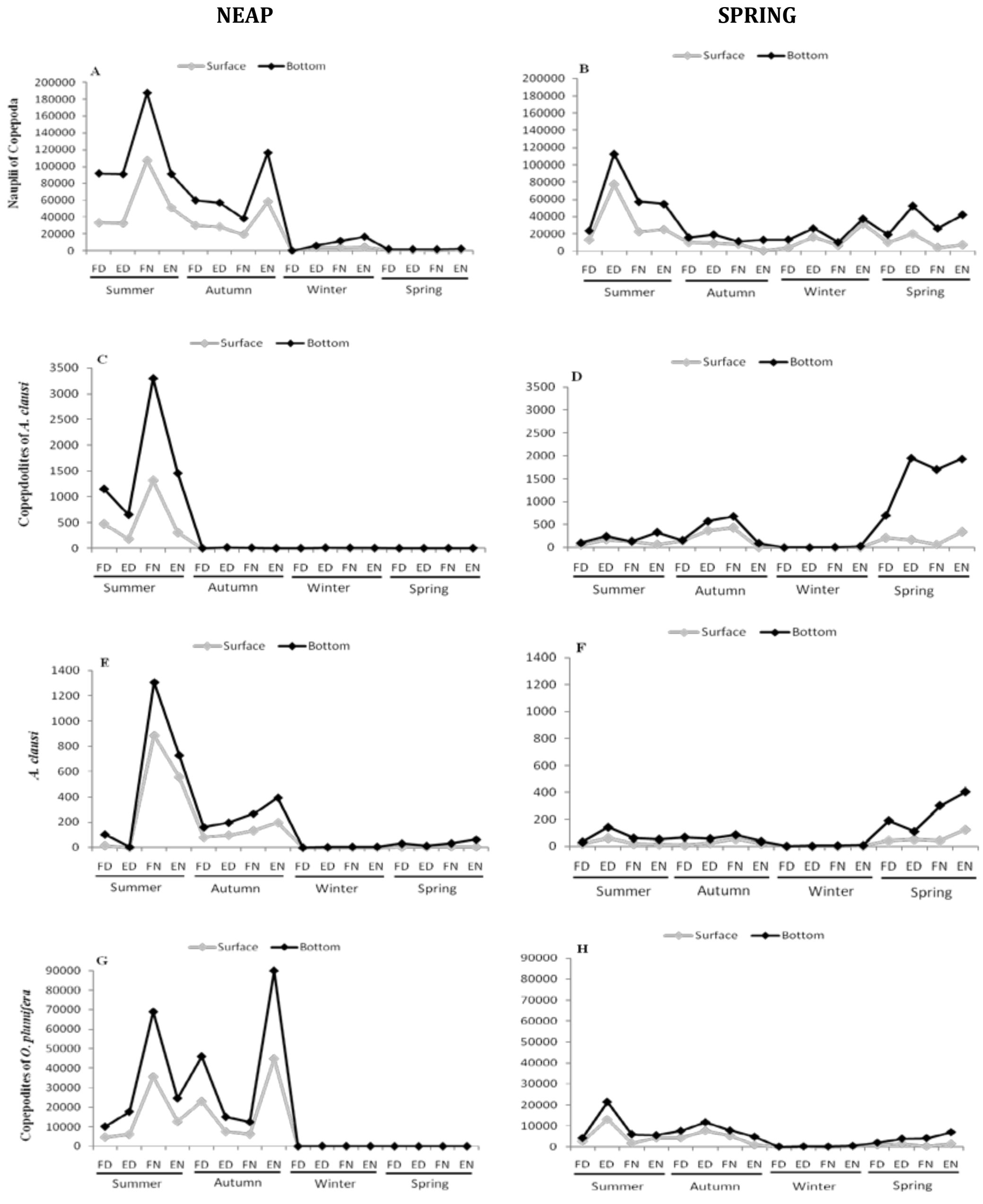


Figure 3 – Seasonal cycle of the dominant marine copepod and the resident estuarine species' densities, and respective life history stages, over neap-spring tides, for each diel and tidal cycles (FD – Flood day; ED – Ebb day; FN – Flood night; EN – Ebb night).

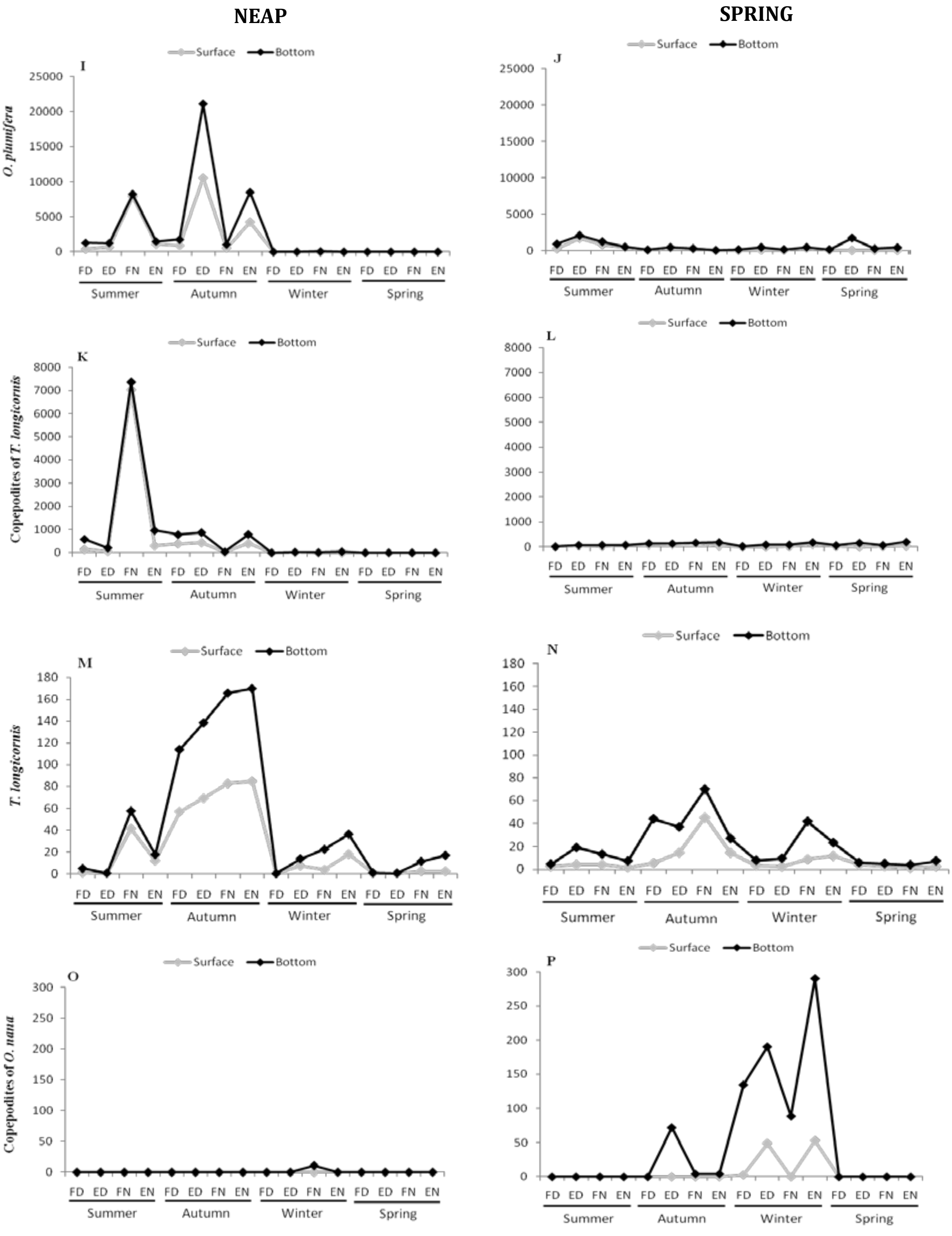


Figure 3 – [continued]

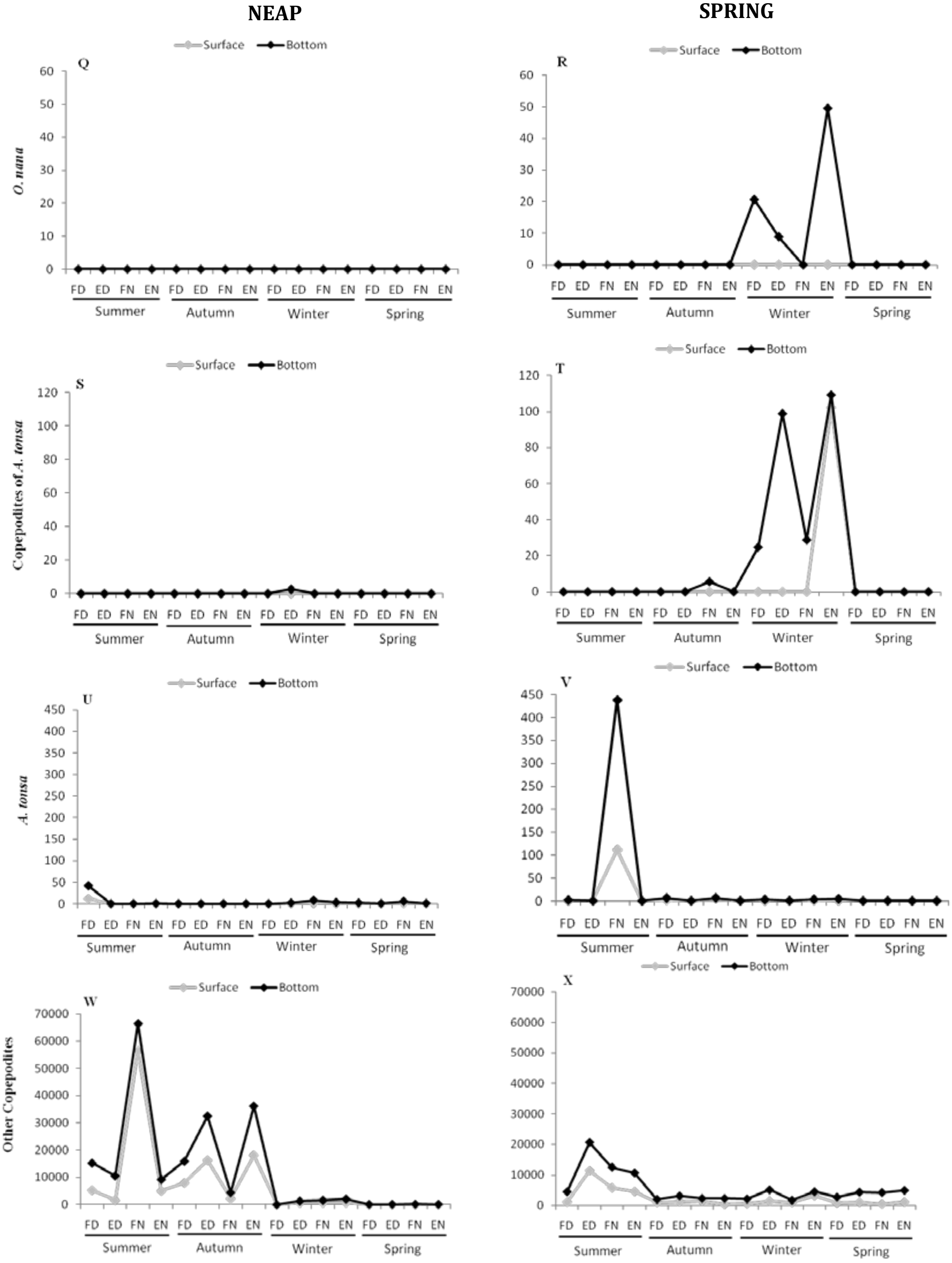


Figure 3 – [continued]

Relationships between environmental factors and copepod assemblages

The factor plots of the first two axes of the compromise analysis are shown for the species assemblages (Fig. 4A – see Table 2 for species codes) and for the environmental variables (Fig. 4B). The eigenvalues diagram (Fig. 4C) shows that the first axis was clearly dominant (accounted for 93% of the explained variance) in contrast with the second axis (3% of the explained variance) which was much less significant. From the species compromise analysis (Fig. 4A), three groups, belonging to two species, were correlated with axis 1: from left to right copepodites of *T. longicornis*, *T. longicornis* and copepodites of *O. nana*. All the other species adult stages and copepodites showed an intermediate position between axis 1 and 2, but may be attached to the horizontal axis. The axis 2 was characterized by *O. nana*, copepodites and adults of *A. tonsa*, nauplii of Copepoda and copepodites of *O. plumifera*. From the environmental compromise analysis (Fig. 4B), the horizontal axis expresses an opposition between POM, salinity and SPM (in the left) and Si, NH_4 and PO_4 (in the right). The axis 2 was characterized by pH. NO_3, like chlorophyll *a* (Chl *a*) concentration, has an intermediate position between axis 1 and 2. However, NO_3 can be associated to the horizontal axis. Temperature, NO_2 and dissolved oxygen concentration (O_2) presented short arrows meaning that they have an average performance in all sampling period. So, they do not play a major role in the average species-environment relationship. Furthermore, Chl *a* concentration is opposed to pH and O_2. The most abundant species in the samples were mainly associated with the same environmental variables: salinity (Sal), SPM and POM (Figs. 4A and 4B). *O. plumifera*, copepodites of *A. clausi* and copepodites of *T. longicornis* were more abundant at higher concentrations of Sal, SPM and POM (and when the nutrients concentrations NO_2, Si, NH_4, PO_4 and NO_3 were low). Copepodites of *O. plumifera*, *A. clausi* and copepod nauplii were clearly influenced by high values of pH and O_2 and lower values of Chl *a* concentration. Copepodites and adults of *A. tonsa*, copepodites and adults of *O. nana* and *T. longicornis* were influenced by warmer waters. Moreover, *A. tonsa* (adults and juveniles) and *O. nana* (adults and juveniles) were the species with lowest densities while *O. plumifera* (adults and juveniles), *A. clausi* (adults and juveniles), *T. longicornis* (adults and juveniles) and other copepodites showed the highest densities (Figs. 4A and 4B).

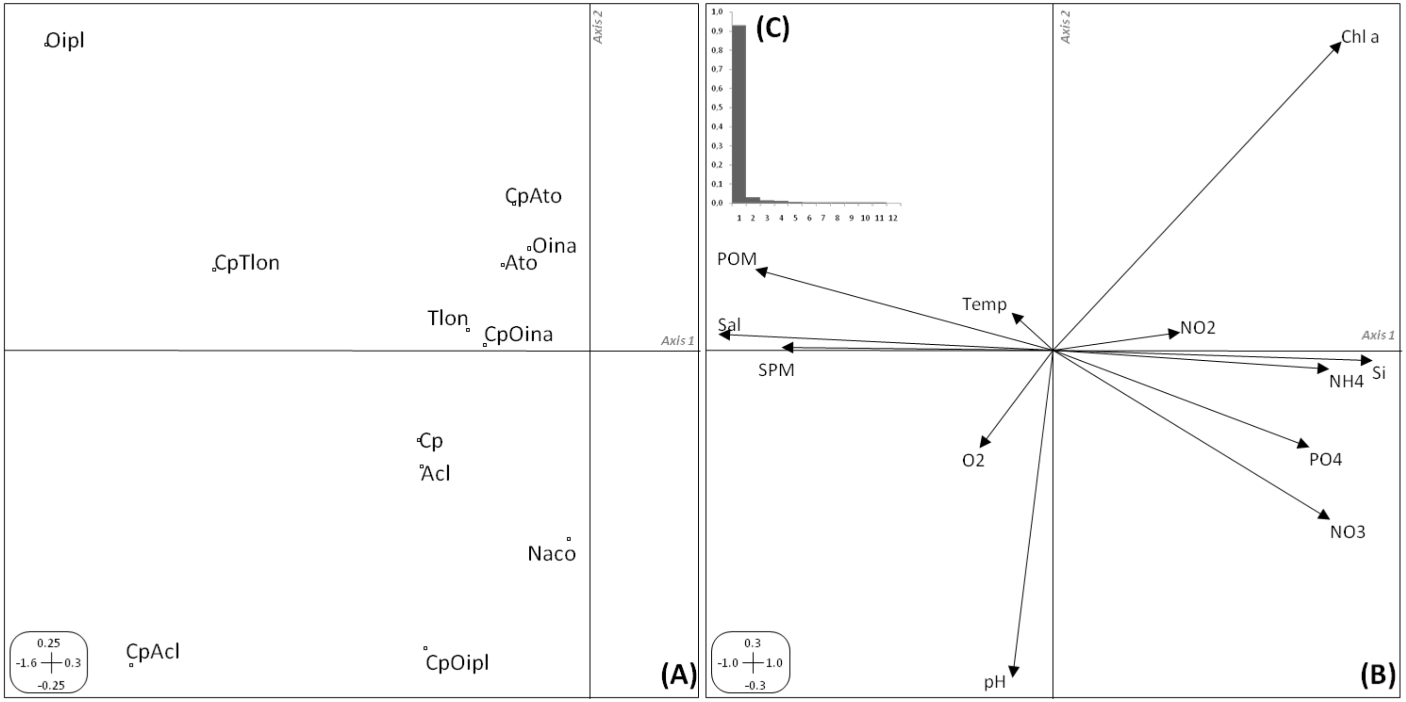


Figure 4 – Compromise factor map of the STATICO analysis of the copepod species variables (A) and environmental parameters (B). (C) Eigenvalues diagram. The scales for axes are given in the boxes. Temp – water temperature, Sal – salinity, O2- dissolved oxygen, PO4 – phosphates, NO2 – nitrites, NO3 – nitrates, Si – silica, NH4 – ammonia, Chl *a* – chlorophyll *a*, SPM – suspended particulate matter, POM – particulate organic matter . See table 2 for species codes.

Table 2 – Code abbreviations of species (including life stages) used in multivariate analysis (STATICO).

Taxa		Abbreviation	Habitat
	Nauplii Copepoda	Naco	
	Other Copepodites	Cp	
Calanoida			
Acartiidae	Copepodite *Acartia clausi*	CpAcl	Marine
	Acartia clausi	Acl	Marine
	Copepodite *Acartia tonsa*	CpAto	Estuarine
	Acartia tonsa	Ato	Estuarine
Temoridae	Copepodite *Temora longicornis*	CpTlon	Marine
	Temora longicornis	Tlon	Marine
Cyclopoida			
Oithonidae	Copepodite *Oithona nana*	CpOina	Estuarine
	Oithona nana	Oina	Estuarine
	Copepodite *Oithona plumifera*	CpOipl	Marine
	Oithona plumifera	Oipl	Marine

Dynamics of copepod species

In figure 5 is the projection of the environmental variables and of the species (see Table 2 for species codes) of original tables on the compromise axes. The graphics has been separated according to seasons and lunar phases. The stable part of the species-environment dynamics, revealed by the compromise analysis, was well expressed in spring at spring tide (SPSPR). Winter at neap (NPWIN) and spring (SPWIN) tides followed this tendency. To highlight the lack of representativeness in the spring neap tide (NPSPR), in comparison with the other lunar condition, most species rise very close to the origin axes, representing the lowest densities. Considering SPSPR, and similarly to the pattern found in the compromise analysis, the estuarine cyclopoid *O. nana* (adults and copepodites) and the estuarine calanoid

A. tonsa (adults and copepodites) showed the lowest densities. Additionally, the species-environmental relationship was very similar with the representation of the compromise analysis. Indeed, at this season case (SPSPR), *A. clausi* (adults and juveniles), *O. plumifera* (adults and juveniles), juveniles of *T. longicornis* and other copepodites were influenced by saline waters, higher values of POM and SPM. Furthermore, copepodites of *A. clausi* and copepodites of *O. plumifera* were positively correlated with higher values of pH while *O. plumifera* and copepodites of *T. longicornis* were influenced by lower values of pH (Figs. 5A and 5B). A similar trend was observed in *O. plumifera* (copepodites and adults) in winter at neap tide (NPWIN). This cyclopoid showed a positive correlation with saline and warmer waters and higher values of SPM and POM. In this season case (NPWIN), nauplii of copepods was positively correlated with pH and negatively correlated with all other environmental parameters. Still, in spring tide (SPWIN) nauplii of copepods were influenced by colder waters and lower values of salinity, SPM and POM, similarly to copepodites of *O. plumifera*. On the other hand, copepodites of *T. longicornis*, copepodites of *A. tonsa* and *O. nana* (adults and juveniles) were influenced by warmer and saline waters, higher values of SPM and POM.

The projection of the samples of the original tables on the compromise axes, in terms of both environmental parameters and species structure is shown in figure 6. Overall, the samples were grouped in the central part of the axes. Exceptions are at neap tide for summer and at spring tide for winter and spring. Spring - spring tide (SPSPR) was the sample that expresses the best consensus (short arrows) between environmental and species structure (which confirms what has been previously described, since this is the situation that best represents the compromise image). An opposition (separated by axis 2) between surface-ebb tide (right) and bottom-ebb tide (left) (SE and BE, respectively) was observed. Still, it was not clear a distinction between day and night. Additionally, the samples surface-flood tide (SF) and bottom-flood tide (BF) were mainly projected in the upper horizontal axis. So, BE samples were mainly associated to the pattern representing the correlation between POM, salinity and SPM with *A. clausi* (adults and juveniles), *O. plumifera* (adults and juveniles), copepodites of *T. longicornis* and other copepodites (as previously described; see Figs. 5A and 5B). Moreover, the SE samples are characterized by a positive correlation (and higher values) of Si, NH_4, PO_4, NO_3 and the low densities of *A. clausi* (adults and juveniles), *O. plumifera* (adults and juveniles), copepodites of *T. longicornis* and other copepodites. In summer (neap tide) and autumn (spring tide), day and night samples were spatially opposed. In summer (NPSUM), night was mainly projected at the left side and in autumn (SPAUT) night was projected mainly at the right side. This means that diel cycle was also an important factor structuring species assemblages. In all other samples, this contrast was present but not so prominent. At spring tide winter (SPWIN), BE and BF samples were mainly project on the second quadrant, meaning that these samples are essentially associated to juveniles of *A. tonsa*, *O. nana* (adults and juveniles) and juveniles of *T. longicornis* and associated with the environmental parameters: salinity, POM, Temp and SPM (see Figs. 5A and 5B). The SF (surface flood tide) samples were mainly projected on the right side of axis 1 being characterized by high densities of nauplii and copepodites of *O. plumifera* (see Figs. 5A and 5B).

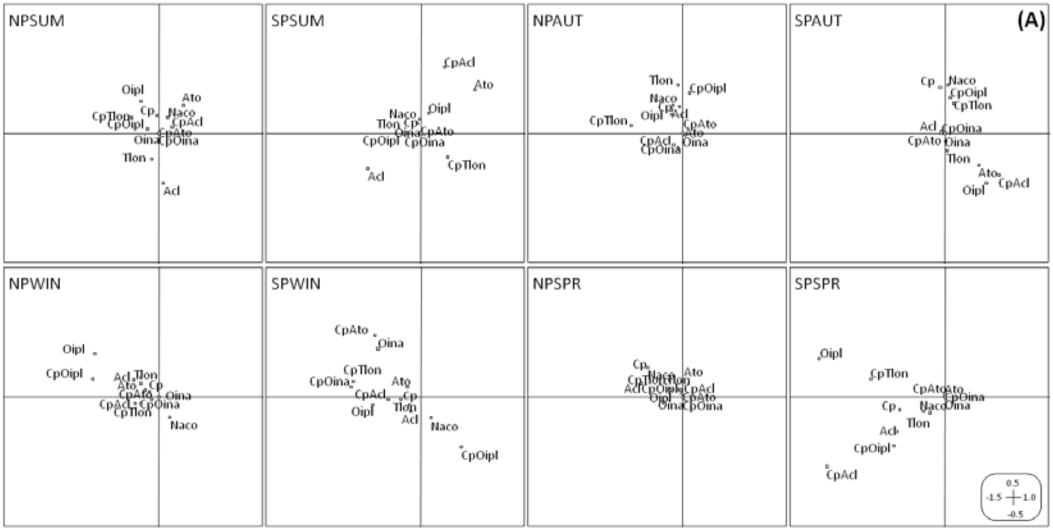


Figure 5 – Trajectories factor maps of the STATICO analysis: seasonal projections (A) of the average position of copepod species and (B) of the environmental variables for each lunar phase (neap and spring tides) at the mouth of the estuary. The scales for axes are given in the boxes. For species codes see table 2. Environmental variables codes as in figure 4.

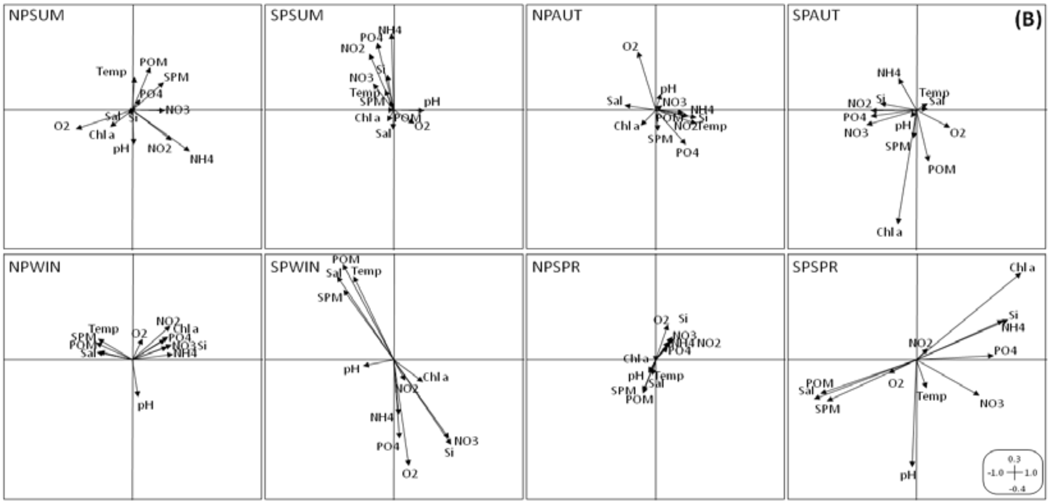


Figure 5 – [continued]

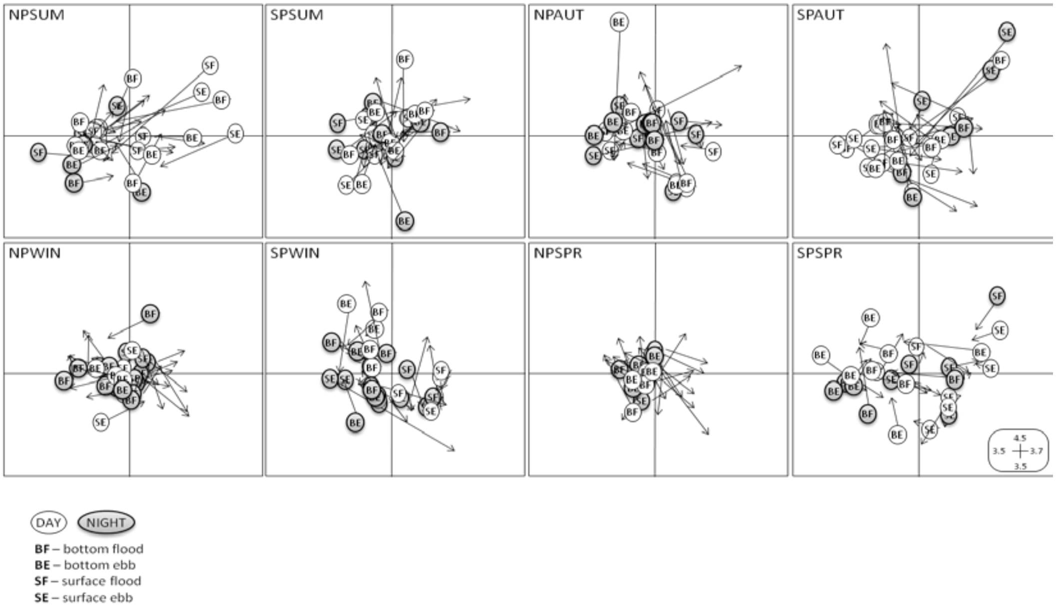


Figure 6 – Trajectories factor plots of the STATICO analysis: projection of the samples in response to two depths ranges (surface and bottom), tidal and diel cycles on the first factorial plan of the compromise analysis, for each season and neap-spring tides. Each sample is represented by two points: one is the projection of the row of the species table (circle: origin of arrows), and the other is the projection of the row of the environmental table (end of arrows). The length of the connecting line reveals the disagreement or the consensus between the two profiles (species–environment), i.e., the length of the line is proportional to the divergence between the datasets. When the datasets agree very strongly, the arrows will be short. Likewise, a long arrow demonstrates a locally weak relationship between the environment and copepod features for that case. The scales for axes are given in the boxes.

Seasonal, lunar and spatial patterns of copepods distribution in the Mondego estuary

The distribution inside the estuary showed that the studied species occurred mainly at downstream stations of both arms of the estuary (St S1 and St N1), with the exception of the resident *A. tonsa* that occurred at upstream stations, more precisely at the south arm station near the Pranto River (St S2) (Fig. 7). A similar pattern was observed in *O. nana* (Figs. 7Q-7S). This species revealed an estuarine behavior with the copepodites occurring along the estuary and the adults clearly distributed in the inner stations of the estuary, mainly at upstream stations (St N2), during autumn. Nauplii of copepods were observed at neap tide, in autumn and spring, at downstream stations (St S1 and N1) and at St N2 (belonging to brackish and freshwater species), whereas at spring tide naupliar stages were predominant in winter at St S2 (Figs. 7A and 7B). A similar distribution was observed in juveniles of *A. tonsa*, at spring tide (Fig. 7M). Adults and juveniles of this species were observed in autumn, at St S2 in neap tide (Figs. 7N and 7P).The highest densities of *A. clausi* (adults) were present in autumn and spring, at downstream stations, while juveniles were dominant in summer and winter at St S1 (Figs. 7C-7F). Juveniles and adults of *T. longicornis* were typically abundant in summer, at downstream stations (St N1 and St S1), at both lunar phases (Figs. 7G-7J). Moreover, adults of *T. longicornis* were also abundant in autumn, at the same sampling stations and lunar phases described above (Figs. 7I and 7J).

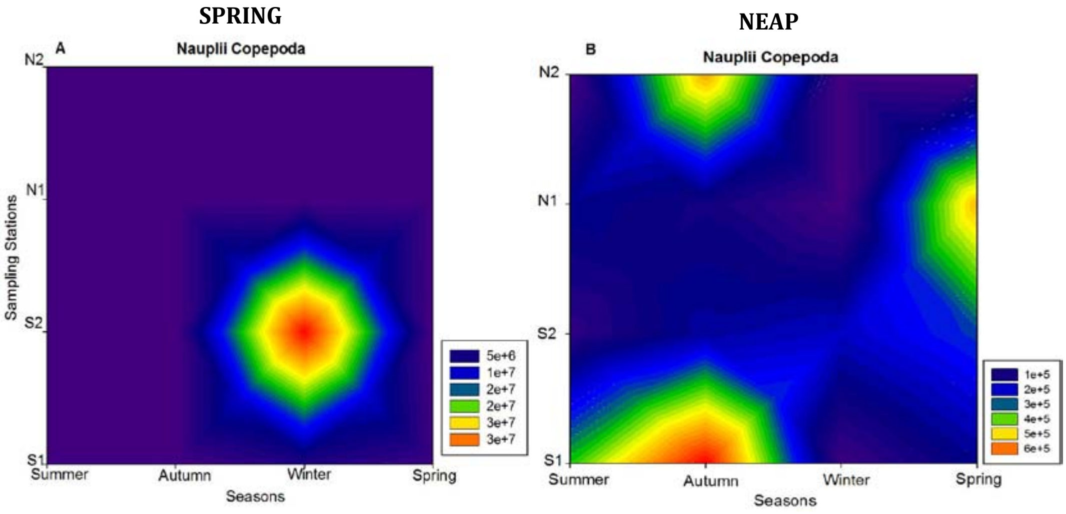


Figure 7 – Seasonal and spatial densities of the copepod species, and their respective life stages, over neap-spring tides, in Mondego estuary.

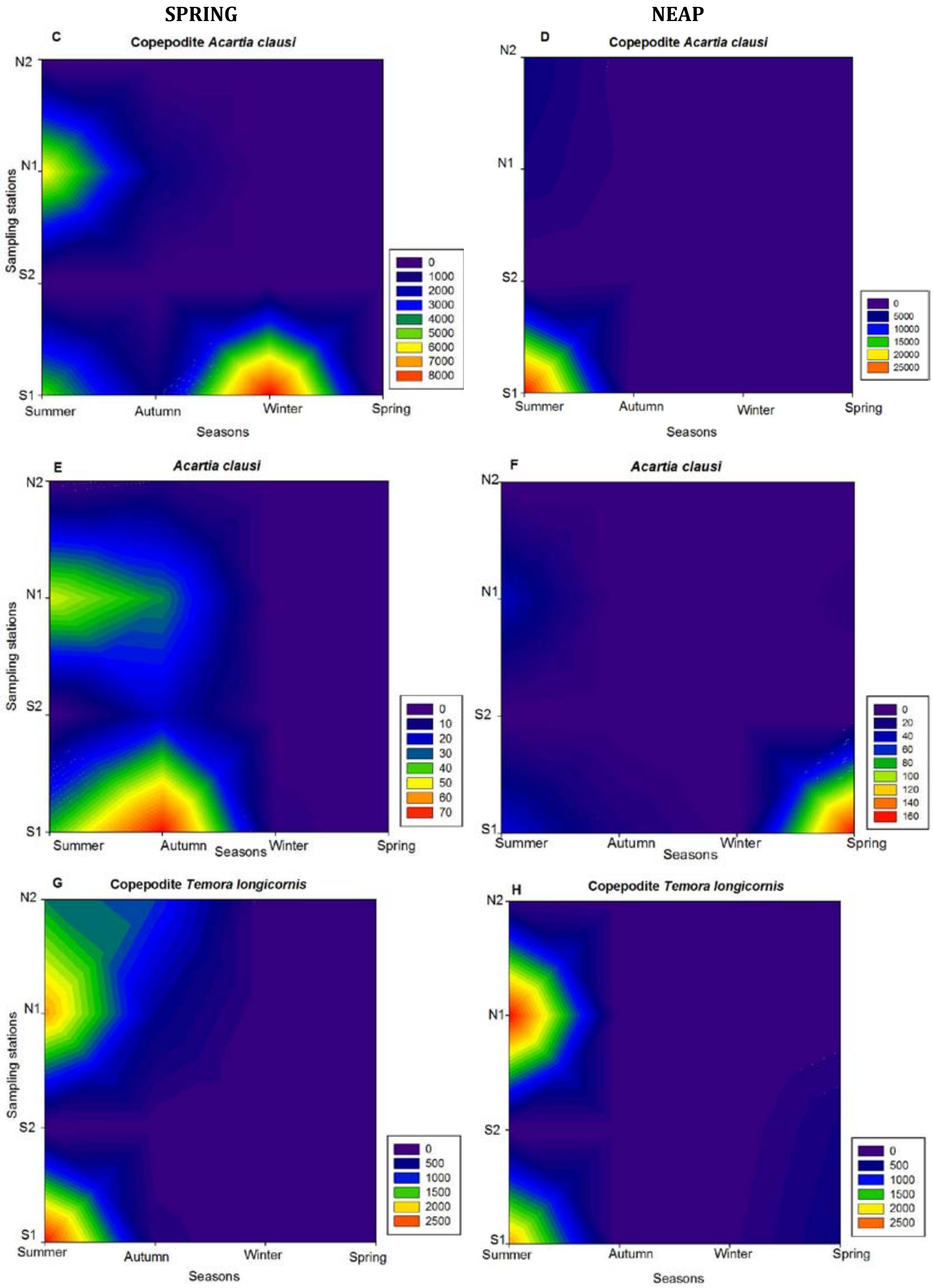


Figure 7 – [continued]

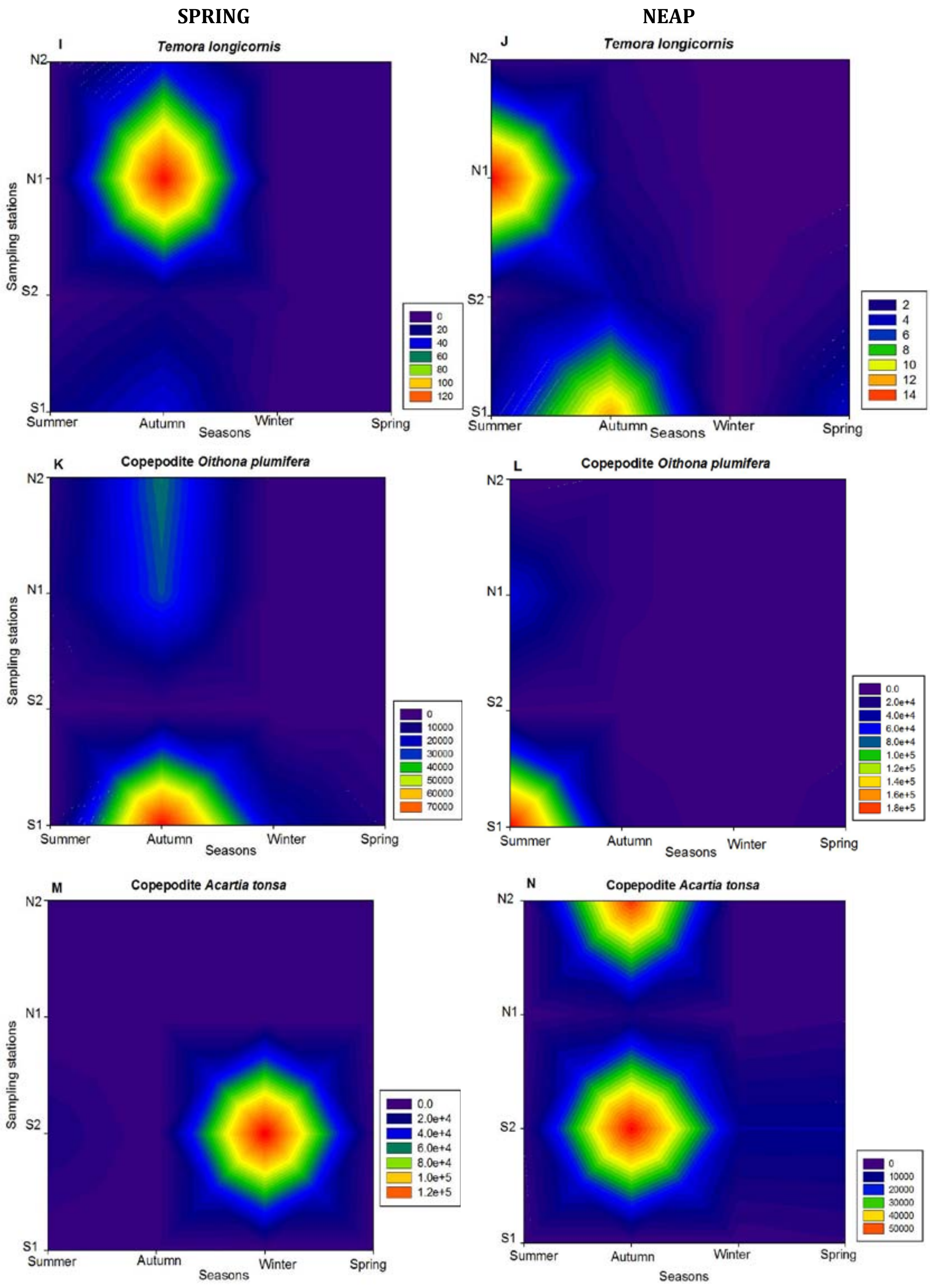


Figure 7 – [continued]

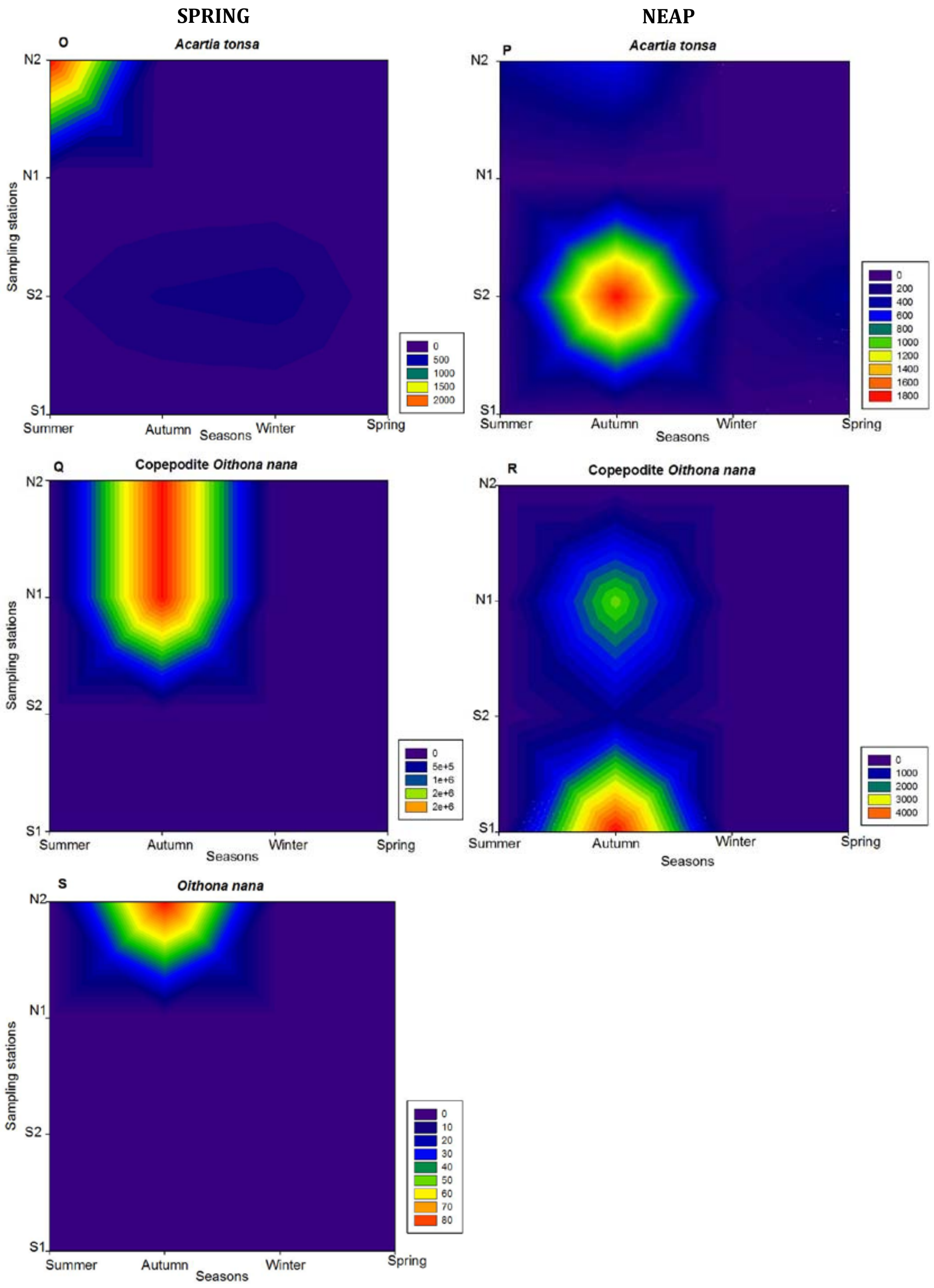


Figure 7 – [continued]

DISCUSSION

Estuarine systems are unstable habitats characterized by large scale seasonal fluctuations and small scale variability (tidal, diel, and water column) of the biological and environmental factors. Previous works had been developed in the study area in order to examine the relationship between environmental factors and small-sized zooplankton distribution (Gonçalves et al., 2010a, b; Vieira et al., 2003). These first studies with the small-sized zooplankton fractions proved that the studies with the mesozooplankton fractions (200 and 335 μm mesh net sizes) (Marques et al., 2007a, b, 2008, 2009; Primo et al., 2009) either under-sampled or missed the overall contribution of this small-sized component, a fact already pointed out by several studies (Gallienne and Robins, 2001; Hopcroft et al., 2005; Satapoomin et al., 2004; Williams and Muxagata, 2006). However, this is the first study encompassing species' life cycles distribution, including smaller specimens, with seasonal, lunar and diel cycles.

All of the main copepod species identified in Mondego estuary occurred mainly at neap tide, with the exception of winter that presented higher densities in spring tide, mainly related to *A. tonsa* and *O. nana*. The pattern of densities observed in this study shows a clear dependence between the structure of copepod community and the lunar cycle. Moreover, the light cycle was also preponderant at summer samples. Similarly, Kennish (1990) stated that the structure of zooplankton community along the estuary depends of both neap-spring and ebb-flood cycles. In the Portuguese coast, neap high tide occurs in the early evening, namely crepuscular phase. Several works (Gonçalves et al., 2003; Paula, 1989; Queiroga et al., 1994, 1996, 1997) performed in estuaries of the Portuguese coast (Mondego estuary, Mira estuary and Canal de Mira) reported the synchronism between the larval-releasing activity of several decapod species with neap high tide during night-time, rather than tidal amplitude. The semi-lunar rhythm of larval released during nocturnal maximum amplitude tides suggests an export strategy in species' life cycles using strong ebb currents to a rapid export to the ocean (Papadopoulos et al., 2002; Zeng and Naylor, 1997). This synchronism behavior between light intensity and lunar phase, minimizes visual predation, hence reducing vulnerability to predators and consequently individual losses. Indeed, in our work spring tide samples were performed at full moon, corresponding to less dark nights, where the lower densities of copepod species were found. Finally, higher densities of copepodites stages were found mostly near the bottom. This pattern should be explained by: 1) the ability of the organisms to adjust their diel and tidal rythms in order to avoid the surface, mainly during the day; and 2) the development of a mechanism to avoid physical stressors such as the tidal currents that transport them outside the system and also to control their horizontal transport, maintaining themselves inside the estuary (Hill, 1991; McLusky and Elliot, 2004; Morgado et al., 2003). An opposite trend was observed to copepod nauplii and copepodites of *O. plumifera* that presented higher densities at surface flood tides. Our findings suggest that nauplii and juveniles of *O. plumifera* do not remain inside the estuary, being carried out to coastal areas by flood tides. In fact, small-sized copepods like *Oithona* and *Oncaea* are referred as the mainly prey of larger predators, including important commercial fishes (e.g. sardines and anchovies larvae stages), having an important role in the dynamics of marine food webs (Porri et al., 2007; Takashi and Uchiyama, 2008).

Titelman and Fiksen (2004) claimed that nauplii of most species reside near the surface and suggested that the distribution of small copepods, especially the nauplii and small species such as *Oithona* spp., differ from the better studied large species and later life history stages. Nauplii and small oithonids are less sensitive to visual predators and more sensitive to invertebrate predators such as larger copepods. Generally, both calanoid and cyclopoid nauplii were found near the surface, while copepodites stayed deeper in the water column (Titelman and Fiksen, 2004).

At the Mondego estuary, neritic species showed preferences by saline waters, distributing mainly at downstream stations and at the mouth of the estuary, whereas resident species distributed mainly at upstream stations. In this way, earlier stages were transported to downstream stations and to the mouth of the estuary, whereas adults returned to upstream stations, the area of adult populations. *O. nana, A. tonsa* and other copepodites showed a similar behavior presenting a clearly estuarine distribution. Our findings exposed a novelty about the behavioral aspects of this Oithonidae species and about the copepodites stages that was not explored yet. In previous works this species (as other smaller specimens and earlier developmental stages) were under estimated due to the usage of larger mesh size nets (Azeiteiro et al., 2000; Marques et al., 2009; Primo et al., 2009). Our findings demonstrated that *O. nana* remains in the estuary, reproducing and performing the development stages inside the estuarine system. Copepodites of *O. nana* showed a wide distribution along the estuary, mainly at downstream stations, whereas the adults prefer brackish waters, occurring at upstream stations. Indeed, some authors (Porri et al., 2007;

Williams and Muxagata, 2006) suggests the sharp spatial distribution of juveniles and adults of *O. nana* to be related to a characteristic of respiratory and feeding rates patterns added to a wide tolerance to salinity and temperature of these stages. Considering *A. tonsa,* this is an important resident estuarine species in Mondego estuary, being a common and dominant species of the upper reaches of the estuary (Gonçalves et al., 2010b; Marques et al., 2008). The ecology of this species is well documented by several authors being considered an important and abundant taxon in European estuaries (Cervetto et al., 1999; David et al., 2007; Tackx et al., 2004).

There is now a growing literature for coastal marine ecosystems on both the numerical importance of cyclopoid copepods, particularly the genus *Oithona*, and also their relative importance compared with calanoid copepods (Gallienne and Robins, 2001; Porri et al., 2007; Turner, 2004; Williams and Muxagata, 2006). This study aids to clarify the ecological significance of this small-sized cyclopoid fraction, namely the numerically significant presence of the estuarine *O. nana* (Williams and Muxagata, 2006), within the upper estuary, with a progressive decrease in abundance towards the mouth of the estuary, complementing the published mesozooplankton studies on the Mondego estuary. This was an already verified distributional pattern for the species in Southampton Water (Williams and Muxagata, 2006), that in the Mondego estuary was clarified with an obvious residence behavior of the species and the more saline waters preference of the copepodites. *O. nana* is a widespread neritic species (Digby, 1950; Lampitt, 1979; Paffenhöfer, 1993; Ryan et al., 1986). It has been extensively reported in estuarine 'small' mesh sampling (<200 μm) (Hopcroft et al., 1998; Reeve, 1975) although, because of its small size, it is consistently underestimated (Gallienne and Robins, 2001; Hopcroft et al., 1998; Reeve, 1975; Williams and Muxagata, 2006).

The structure of Copepod species' distribution in the Mondego estuary proved to be best correlated with environmental factors registered in spring. Other interesting result verified in this study was that depth and tide currents were the main physical conditions under which the vertical migration patterns resulted at horizontal displacement or retention of copepod species, while day and night were not ecologically significant, unless in summer.

This work contributes to a further knowledge of the distribution and life history stages of the main copepod community, associating them with physical factors (e.g. lunar phase, diel cycles, depth, tidal currents), in order to identify the factors that contribute to vertical migratory behavior promoting retention or displacement in the estuarine system.

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CHAPTER IV

Spatial and temporal distribution of harpacticoid copepods in Mondego estuary

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ABSTRACT

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 6 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 at downstream areas. 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 (St 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, characterised 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.

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 implies also 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 (Richmond et al., 2007; Roff 1992). Especially in a changing environment like an estuary, it is essential to understand the natural degree of variation of copepod communities.

In general, copepods dominate mesozooplankton communities (Cornils et al., 2007; Kršinić and Grbec, 2002). Among copepods, cyclopoids usually dominate the summer and autumn assemblages, whereas calanoids are predominant in winter and spring (Calbet et al., 2001; Cornils et al., 2007; Kršinić and Grbec, 2002). Indeed, copepod nauplii are the 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; Cornils et al., 2007; Kršinić and Grbec, 2002), this order constitutes a diverse group and the second most abundant in what respect to meiofauna taxon (Huys and 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 and 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. Despite 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; Cornils et al., 2007; Kršinić and Grbec, 2002; Uriarte and Villate, 2004, 2005).

Despite several studies were based on the composition of marine harpacticoid fauna (Huys et al., 1996; Lang, 1948, 1965a, b), they were restricted to a limited number of habitats or specific areas such as the Gulf of Mexico and other tropical areas (Fiers, 1995, 1996; Fleeger and Clark, 1979; Rutledge and Fleeger, 1993; 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; Morgado, 1997; Noodt and Galhano, 1969).

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 (Cardoso et al., 2008; Dolbeth et al., 2007; Verdelhos et al., 2005). 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 (40º08'N, 8º50'W) consists of two arms – north and south, with different hydrological characteristics, separated by Murraceira island (Fig. 1). The northern arm with a lower residence time (<1 day), is deeper (4-8 m at high tide, tidal range 1 to 3m) 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 to 3m), 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 throughout the northern arm (Cardoso et al., 2004; Pardal et al., 2000). 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 economical growth at the regional scale.

Since the late 80's, 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 sub-system 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).

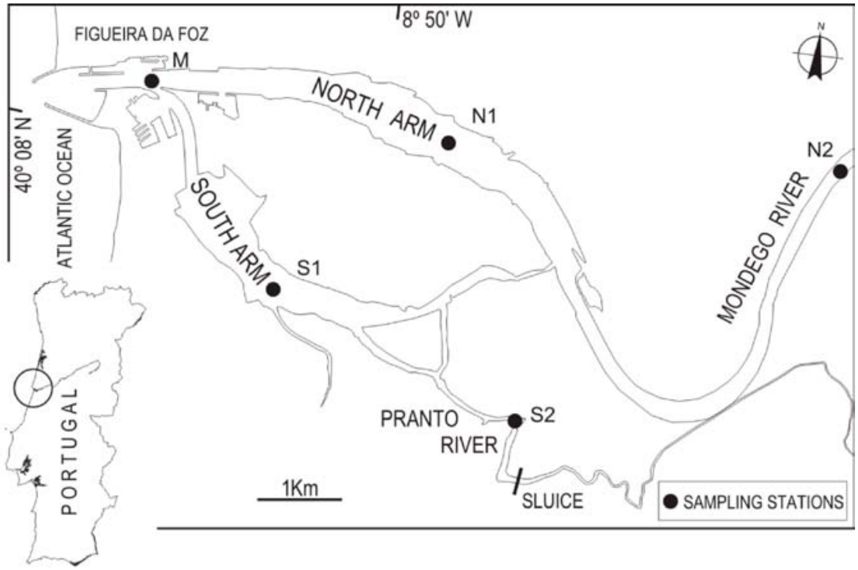

Figure 1 - Map of the Mondego estuary, located on the western coast of Portugal. Sampling stations are indicated (M – mouth station, N1 and N2 – northern arm stations, S1 and S2 – southern arm stations).

Sampling

Harpacticoid samples were collected monthly from February 2005 to January 2007 at five stations of Mondego estuary (Fig. 1, M: mouth of the estuary; N1 and N2: northern arm of the estuary; S1 and S2: southern arm of the estuary), situated in both arms of the estuary in order to cover a maximum and representative area of the system. Sampling was carried out during high tide, with a 63 µm mesh net, with 0.30m mouth 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).

Laboratorial procedures

Standard methods were followed to determine nutrient concentrations (Limnologisk Metodik, 1992 – for ammonia and phosphate; Strickland and Parsons, 1972 – for nitrate and nitrite). Chl *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 ºC for 72 h and combustion at 450ºC for 8h (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, b), Huys et al. (1996), Boxshall and 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 PRIMER statistical package (Clarke and Warwick, 2001) was performed in order to group harpacticoid species with similar distribution and occurrence. 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 version 4.5 package (ter Braak and Smilauer, 1998), on both matrixes where columns were the taxonomic groups and environmental parameters, respectively, and rows of the seasonal data, which were estimated by averaging the monthly values on 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 climate normal 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 (Fig. 2). Nevertheless, precipitation was lower (2005-2006: 855.9mm and 2006-2007: 866.5mm) than the normal precipitation average (1016 mm).

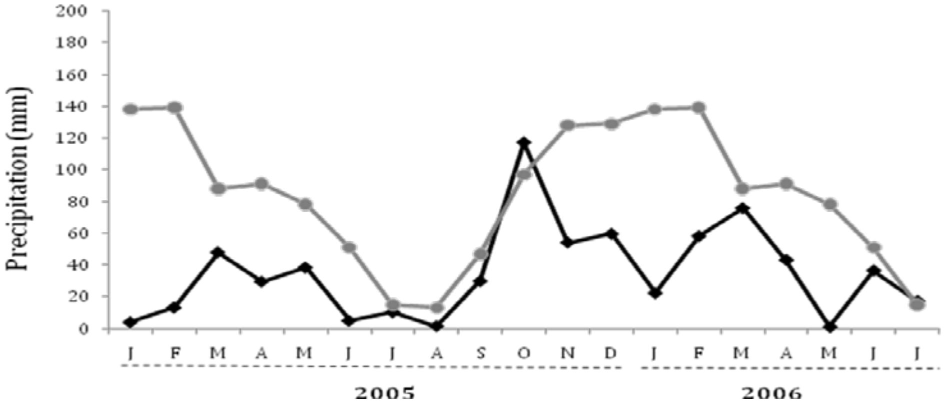

Figure 2 – Monthly precipitation (mm) in Mondego estuary during the study period (2005-2006 and 2006-2007). Grey filled circles represent monthly average of 1961 to 1990 (http://snirh.inag.pt).

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).

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 suspensed 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) and 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 (ºC)**	**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

In upstream stations an increase of Chlorophyll *a* (Chl *a*) concentration was observed, especially in spring and summer, at the north arm station, and at all seasons, except in spring, in the south arm station (e.g. station N2: 10.48 at spring and 11.11 mg.m^{-3} at summer; station S2: 18.97 at winter and 17.33 at summer; and 14.69 mg.m^{-3} at 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 in N1 station (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 ºC to 24.26 ºC, 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, N2 showed the lowest values of salt (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 6 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

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 to 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 *E. acutifrons*, *Microsetella* sp., *Ectinosoma* sp., *H. obscurus*, *T. discipes*, *M. ignea*, *Mesochra* sp. and *P. nanus*. Morgado (1997) found *T. discipes* and *E. acutifrons* as most abundant species.

A cluster analysis (Fig. 3) showed the affinity between species/genus identified in the five stations of Mondego estuary. Group I is composed by the most related species, *Metis* cfr. *ignea* and *Tachidius discipes*, due 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 to the other sampling sites (see Table 1). Group II is the most representative, being composed by 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 2007 and 2005 and autumn 2006, respectively. Group IV was composed of rare species, occurring in one or two sampling stations, in a specific season: *Canuella* sp., *P. viguieri, M. norvegica* (juveniles) and *M. gracilis* (juveniles) occurred mostly in winter in the northern arm stations; *M. norvegica* (juveniles) and *E. melaniceps* appeared in summer in the south and north arm stations, respectively; *L. brevicornis* appeared in autumn in the southern arm of the estuary. Still, the species of this group showed no affinity among them nor between the species of the other groups.

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	*Paronychocamtpus nanus* Sars, 1980	Pana	20.16
		Copepodite *Paronychocamtpus nanus*	CpPana	13.88

A RDA analysis (Fig. 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 relatively 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* (incl. 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.

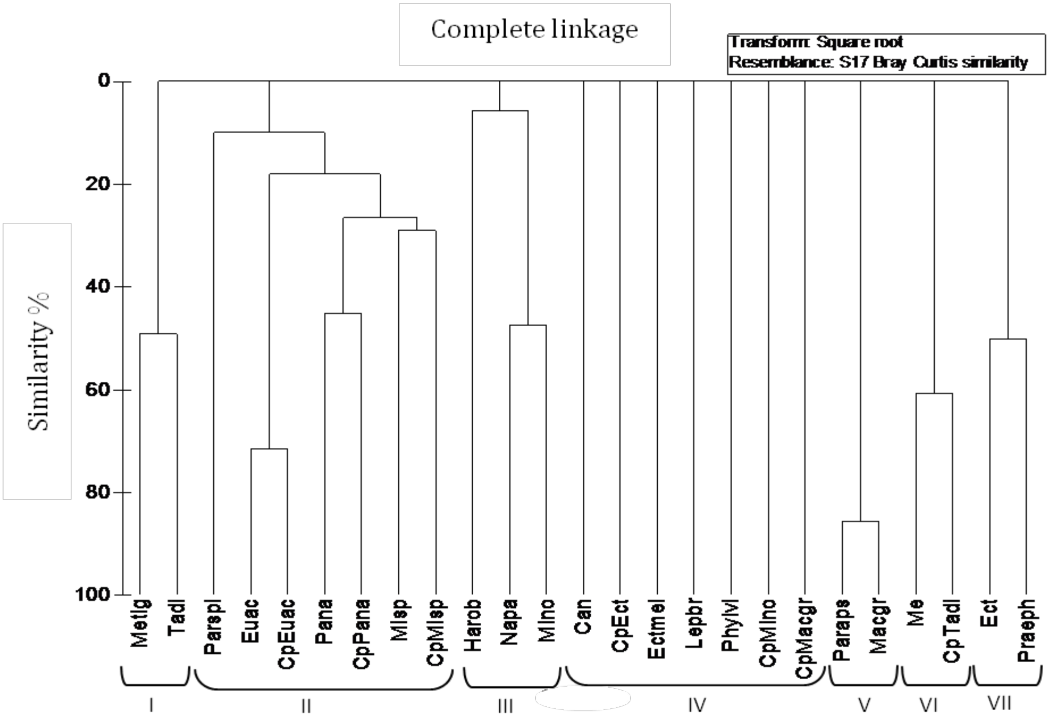

Figure 3 – Cladogram (cluster analysis) grouping harpacticoid species-genus groups based on their distribution in different stations. The seven species-groups of Harpacticoida are indicated by roman numbers.

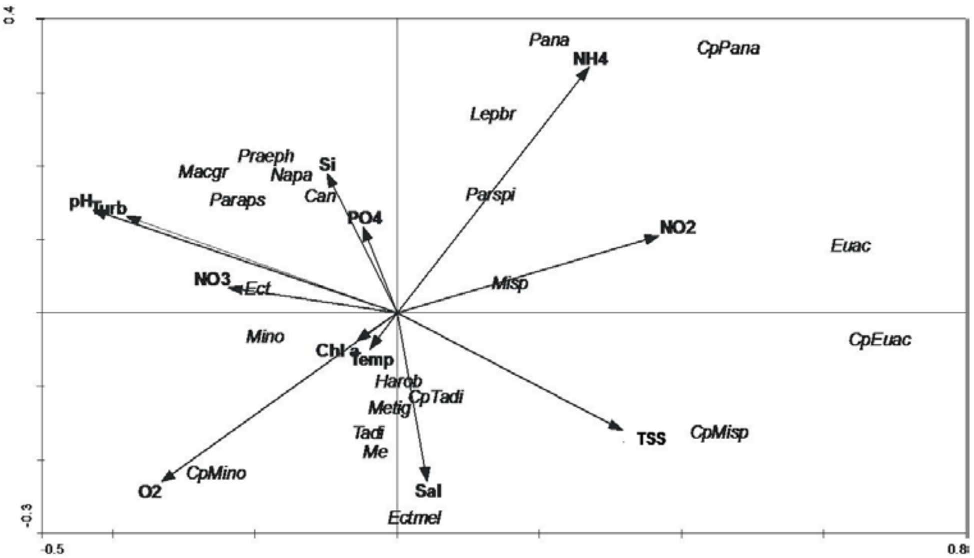

Figure 4 – Results of RDA analysis. Relationships between harpacticoid species distribution and environmental variables in Mondego estuary, from February 2005 to January 2007.

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. Especially the northern arm of the estuary was characterised by a high density of harpacticoids during the whole year, with the highest abundances in station N2 (Fig. 5).

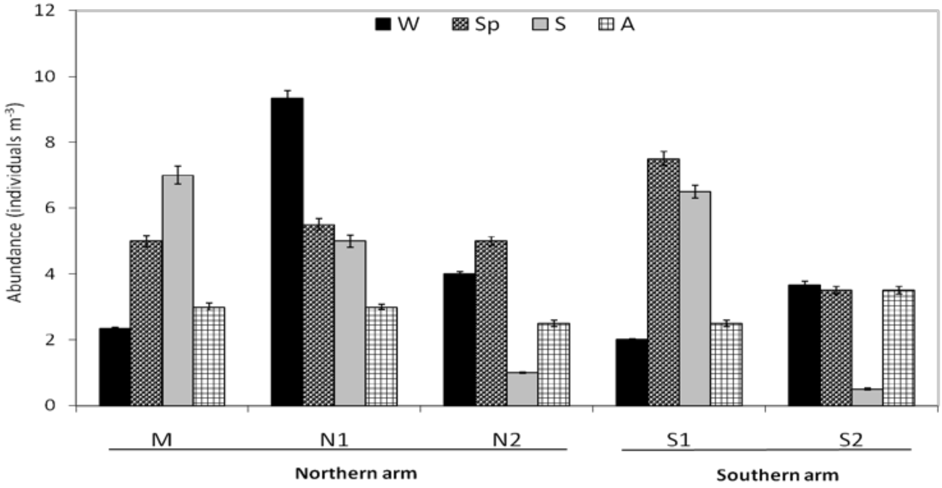

Figure 5 – Average harpacticoid copepod densities (± standard error) in the different seasons and different stations (M- mouth station; N1 and N2– northern arm stations, S1 and S2 – southern arm stations).

More harpacticoids (copepodites and adults) were found in the northern and southern arms (Figs. 6 and 7). Copepodites and adults of *Euterpina acutifrons* and *Paronychocamptus nanus* were dominant depending on the season. In general, *P. nanus* dominated in winter (W) and spring (Sp) seasons, except in spring at M, N1 and S1 stations, which were dominated by *E. acutifrons*. *Paraleptastacus* cfr. *spinicauda* showed an occurrence of 5-10% at all stations, except for station M, where it was observed no distribution.

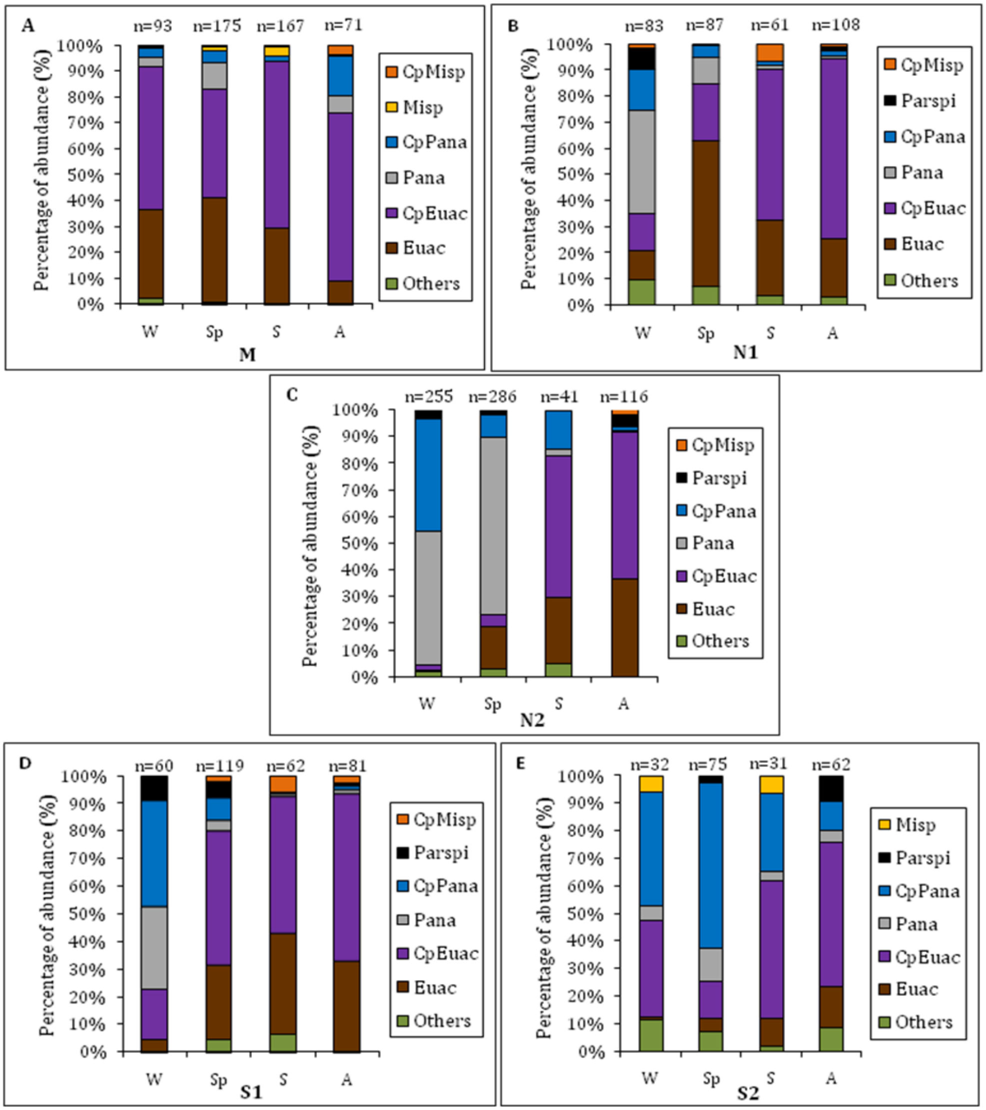

Figure 6 – Relative abundance (%) of harpacticoid species/genera in each sampling station (A – Mouth station; B – N1 station; C – N2 station; D – S1 station; E – S2 station), during seasonal period (W – Winter, Sp – Spring; S – Summer; A – Autumn).

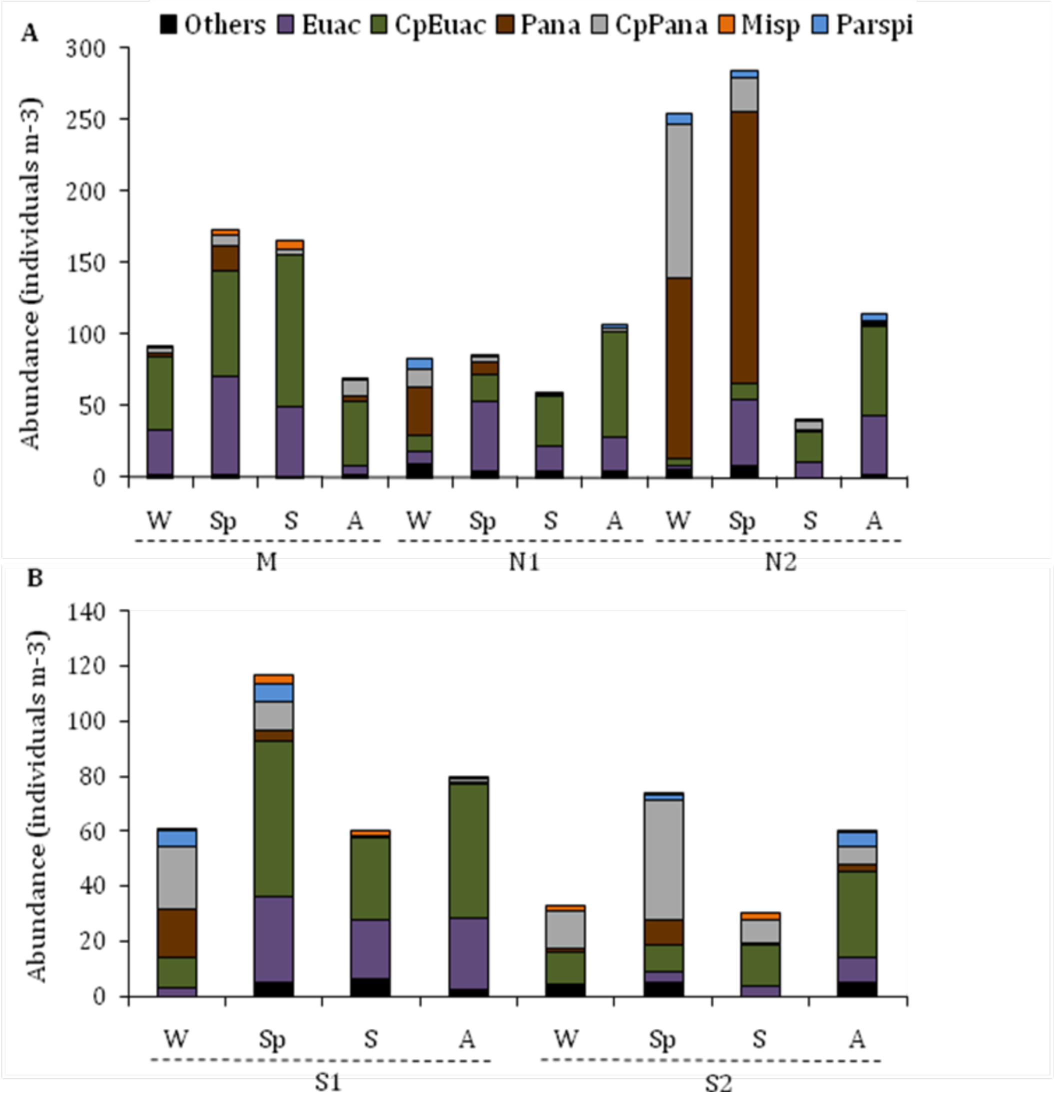

Figure 7 – Seasonal pattern of the composition of harpacticoid copepods community in (A) northern and (B) southern arms of the Mondego estuary.

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 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 *M. 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 (Uriarte and Villate, 2005; Villate, 1997; Walters and Bell, 1994). The deepness of the sampling sites is not below 2 m, achieving 8 m in the north arm. In addition, the sampling occurred always at high tide, when tidal range was at maximum, as stated above.

Uriarte and 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 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 (Roman et al., 1993; Siokou-Fragou and Papathanassiou, 1991). In general, inter-estuarine comparisons showed that copepod abundances were higher in Urdaibai estuary at high salinities, with *E. acutrifrons* being abundant at all salinities and meiobenthic harpacticoids only at the stations with salinities of 31 and 34 psu. Some copepod species show clear seasonal patterns of dominance or differences between estuaries or stations. For instance, *P. parvus* and *A. 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. Still, *O. media* and *T. stylifera* dominated in summer with no significant differences between estuaries or salinity stations, while *P. elongates* 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 and Villate, 2005). *E. 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.

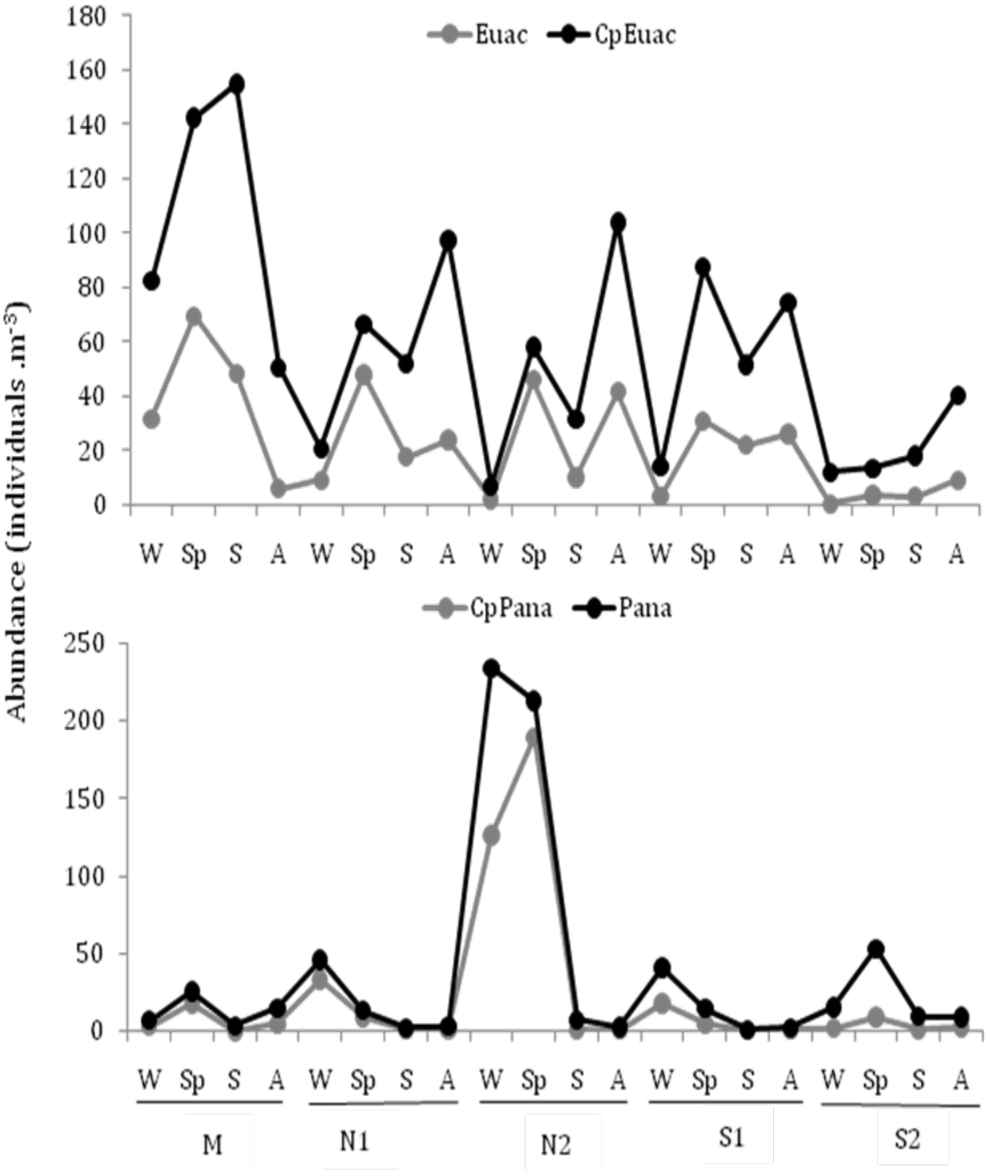

Figure 8 – Seasonal cycle of the most abundant harpacticoid copepods species (*E. acutifrons* and *P. nanus*) vs. neritic-oceanic species (*Macrosetella gracilis* and *Microsetella norvegica*). February 2005-January 2007.

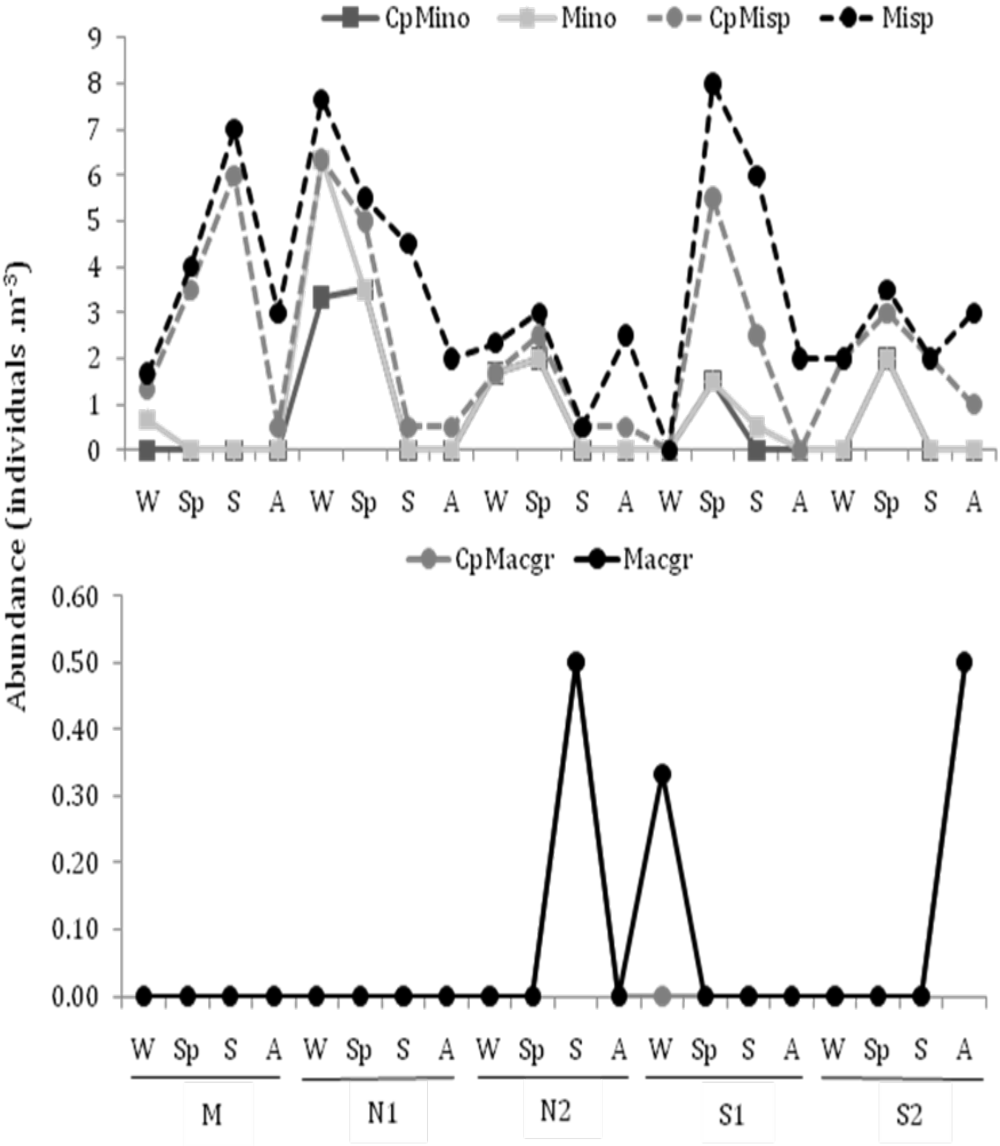

Figure 8 – [continued]

Kršinić and Grbec (2002) studied the distribution of small zooplankton at two stations in Otranto Strait (Eastern 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 observed, unlike to other groups found, no 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 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 (northwest Meditterranean) within an annual cycle. Sampling included an oblique and vertical tows made with a 200 μm and a 53 μm net, correspondingly. Despite of the 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 and Halsey, 2004; Suárez-Morales et al., 2006). In some cases, these copepods are associated with other organisms (e.g. polychaeta) since they can benefit of the fact that 1) the harpacticoids are less subject 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 and Eckman, 1988). Accordingly, species exhibit a variety of morphological characteristics and may be found migrating from different habitats. Still, different forms may 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 would 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 (Buffan-Dubau and Carman, 2000; De Troch et al., 1998, 2005), 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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CHAPTER V

Species-specific fatty acids profiling reveals functional shifts in zooplankton in a temperate estuary

Chapter V is submitted in the form of a manuscript to Hydrobiologia.

ABSTRACT

Fatty acids composition of copepod and cladoceran species in the Mondego estuary (southern Europe) was investigated together with their dietary preferences. These data were used to explain the seasonal variation of the small copepods *Acartia clausi*, *Acartia tonsa*, *Copidodiaptomus numidicus*, *Temora longicornis* and the freshwater cladoceran *Daphnia longispina*. Together with some zooplankonters that were sampled only in a particular season, a total of 12 zooplankton species (7 marine, 2 estuarine and 3 freshwater species) were studied. A multivariate analysis revealed a clear seasonal distribution of zooplankton species in terms of fatty acids composition and abundance, with winter and spring zooplankton species showing maximal concentrations and diversity of total fatty acids. These findings underline the role of lipids as storage during the latter seasons. Estuarine and freshwater species contained a more diverse content of saturated and unsaturated fatty acids rather than the marine species, except for *Centropages typicus*. Fatty acids markers of trophic position indicated the presence of two trophic levels: copepod species are primarily omnivorous, whereas cladocerans showed to be herbivorous. Our results suggest feeding preferences of plankton change spatially and temporally, reflecting the shifts in dominance between diatoms and flagellates as well as between dinoflagellates/diatoms and small animals.

KEYWORDS

Fatty acids, Copepods, Cladocerans, Mondego estuary, Portugal.

INTRODUCTION

Estuaries are transition zones between rivers and the sea and differ from both in terms of biotic (e.g. predation, competition) and abiotic conditions (e.g. temperature, salinity, food quantity and quality), reaching more fluctuations than in marine or freshwater systems (David et al., 2005; Isari et al., 2007). Plankton (and mainly zooplankton) is known to be particularly sensitive to these variations because it is strongly influenced by climatic features and changes in hydrological conditions, resulting in communities that are continuously changing and adapting to environmental factors (Ara, 2001; Beaugrand et al., 2000). This also suggests that plankton is well adapted to changes in the available resources. Copepods are the dominant group of mesozooplankton, followed by cladocerans, playing a key role in the food web as they form a link between primary producers and secondary consumers (Guschina and Harwood, 2009; Richmond et al., 2007). There are important differences between both zooplankton guilds, especially regarding their impact on the lower trophic levels, either directly via feeding or indirectly by influencing nutrient cycling (De Mott, 1995; Hessen and Lyche, 1991). Therefore, determination of biochemical composition of copepod and cladoceran communities has become important to understand their physiological functions, metabolism and nutritive value, as this is very relevant for the energy transfer in aquatic ecosystems and secondary production. Fatty acids (FAs) are one of the most important molecules transferred across the plant-animal interface in aquatic food webs, having an enormous influence on the integrity and physiology of living organisms (Dalsgaard et al., 2003). Moreover, the so-called essential compounds like highly unsaturated fatty acids (HUFA) of n-3 fatty acids (e.g. eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA)), play a key role in the health and function of all animals at all trophic levels, including plankton invertebrates, fish and humans. These HUFAs cannot be synthesized *de novo*, or at least not in sufficient amount for somatic growth, reproduction and survival by consumers (Arts et al., 2009; Wacker and Von Elert, 2001). Furthermore, lipids components are very sensitive to stressors and environmental changes (Arts et al., 2009). Thus, large-scale processes such as eutrophication and global warming may act either independently, or together, leading to an overall decrease in HUFA production in aquatic ecosystems with possible negative implications for the surrounding terrestrial communities. In the last decades the interest on fatty acid composition of aquatic organisms has been increasing. Marine zooplankton, mainly marine copepod species, is a well-documented group due to their use as live feed for commercial species, mainly marine fish (Arts et al., 2009; Dalsgaard et al., 2003; Perumal et al., 2010). However, the link between fatty acid profiles and the seasonal variation of plankton species in the field is still underdocumented.

The current knowledge on the zooplankton in Mondego estuary (Portugal) is a clear example of this type of gaps in our current knowledge. Several studies have been carried out in the Mondego estuary concerning the effects of eutrophication on benthic populations' structure and distribution (Cardoso et al., 2004; Pardal et al., 2000; Verdelhos et al., 2005), and on zooplankton distribution and dynamics (Azeiteiro et al., 1999; Gonçalves et al., 2010a, b; Primo et al., 2009). Despite the extensive literature on the Mondego benthic and pelagic ecology, a more functional approach to trace spatio-temporal changes in the dietary quality and the contribution of zooplankton in energy transfer in the food web is lacking so far. Moreover, no information concerning this issue was yet performed in southern European estuaries. Therefore, it remains unclear how structural changes in species composition are linked to functional changes in species or in species response to environmental changes. Up-to-date tracing techniques like fatty acid profiling can contribute to answer these questions. Moreover, a better knowledge on the ecosystem functioning is crucial to predict the potential impact of future environmental changes. Hence a first attempt was made to determine and compare the diet on fatty acids composition of 12 zooplankton species (7 marine, 2 estuarine and 3 freshwater species) collected in different stations and different seasons. The aim of this study is to focus on the characterization of zooplankton species fatty acid (FA) composition in the mouth and in both arms of the Mondego estuary in order to 1) unravel dietary preferences of zooplankton species in relation to potential food sources and to 2) analyse spatial and temporal (seasonal) patterns in these FA profiles.

MATERIALS AND METHODS

Study site

The Mondego estuary is a small mesotidal system covering an area of 8.6 km^2 along the west Atlantic coast of Portugal (40º 08´ N, 8º 50´ W) (Fig. 1). It comprises two channels, so-called north and south arms, separated by the Murraceira island at about 7 km from the shore and joining again near the mouth. These two arms represent different hydrological characteristics. The north arm is deeper (4-8 m during high tide, tidal range about 2-3 m), has a low residence time (<1 day) and is the location of the commercial harbour and the main navigation channel. The south arm is shallower (2-4 m deep, during high tide), has higher residence times (2-8 days) and the water circulation is mainly dependent on the tides and on the freshwater input from a small tributary, the Pranto River. Freshwater discharge of this river is controlled by a sluice according to the water needs of the Mondego valley rice fields.

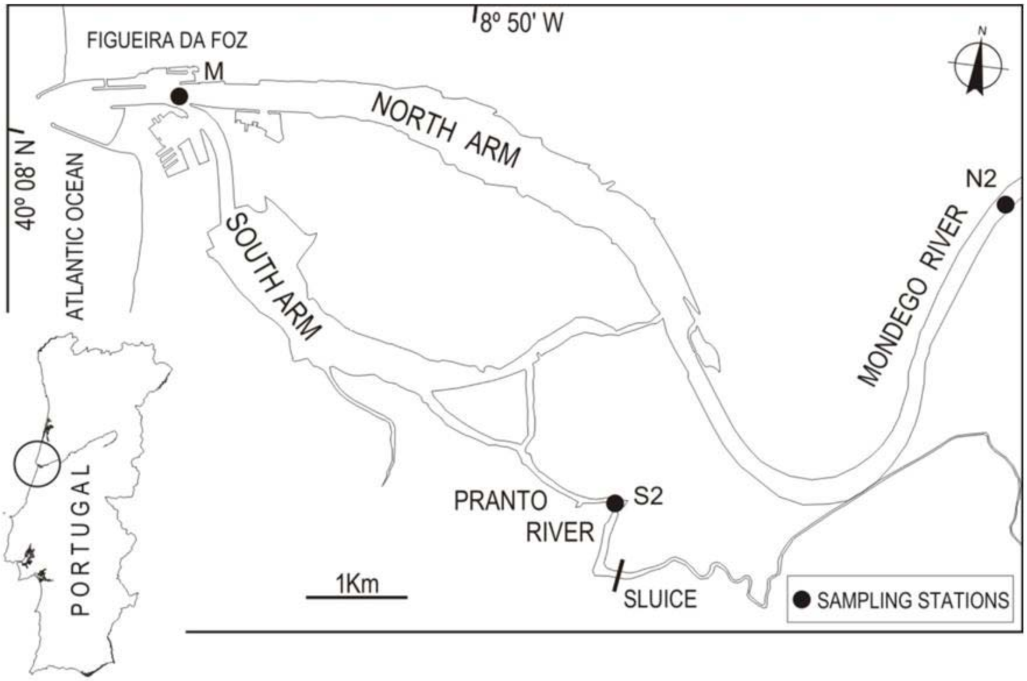

Figure 1 - Map of the Mondego estuary, located along the west coast of Portugal. Sampling stations are indicated (M- mouth station; S2 – southern arm station; N2 – northern arm station).

Sample collection and laboratory analyses

In the Mondego estuary, seasonal sampling (Winter – 28th January; Spring – 26th April; Summer – 15th July; and Autumn – 28th October) of zooplankton was performed, during 2010, in three stations (St M – mouth station; St N2 – station of the north arm; St S2 – station of the south arm) (Fig. 1). Samples were collected by horizontal subsurface tows (bongo net: mesh size 335 µm, mouth diameter: 0.5 m), equipped with a Hydro-Bios flowmeter. Zooplankton samples were brought to the lab, sorted alive at species level, concentrated on GF/F Whatman filters (25 mm diameter) and stored frozen at -80 ºC in eppendorfs. For each species, 3 replicates containing 60 individuals each were collected. Water samples were collected and filtered on GF/F Whatman filters (25 mm diameter) in each sampling station, at each sampling occasion (season) in order to quantify potential food sources. The extraction of total lipids of copepods and cladocerans and methylation to fatty acid methyl esters (FAMEs) for FA analysis was achieved by a modified one-step derivatisation method after Abdulkadir and Tsuchiya (2008). The boron trifluoride-methanol reagent was replaced by a 2.5% H_2SO_4-methanol solution since BF_3-methanol can cause artefacts or loss of PUFAs (Eder, 1995). The fatty acid Methylnonadecanoate C19:0 was added as an internal standard for the quantification (Fluka 74208). Samples were centrifuged (eppendorf Centrifuge 5810R) and vacuum dried (Rapid Vap LABCONCO). The FAMEs thus obtained were analysed using a Hewlet Packard 6890N GC with a mass spectrometer (HP 5973). All samples were run in splitless mode, with a 5µL injector per run, at an injector temperature of 250 ºC, using a HP88 column (60m × 25mm i.d., Df = 0.20; Agilent J&W; Agilent Co., USA) with He flow rate of 1.3 ml min^{-1}. The oven temperature was programmed at 50ºC for 2 min, followed by a ramp at 25ºC min^{-1} to 75ºC, then a second ramp at 2ºC/min to 230ºC with a final 4 min hold.

FAMEs were identified by comparison with the retention times and mass spectra of authentic standards and available ion spectra in WILEY mass spectral libraries, and analysed with the software Agilent MSD Productivity ChemStation.

Quantification of individual FAMEs was accomplished by the use of external standards (Supelco™ 37 Component FAME Mix, Supelco # 47885, Sigma-Aldrich Inc., USA). The quantification function of each individual FAME was obtained by linear regression applied to the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 250 µg mL^{-1}).

Data analysis

The FA profiles of zooplankton were reported for each season and sampling station, over a spatio-temporal scale, by determining their total (mg/ind) or relative (%) concentrations. Multivariate statistical analysis was carried out using PRIMER-6 software (Clarke and Gorley, 2006) in order to examine the variation in FA composition through non-metric multidimensional scaling (n-MDS) plots. The data were converted into similarity triangular matrices using a Bray-Curtis resemblance measure (Clarke and Warwick, 2001). One-way analysis of similarity (ANOSIM) was used to test differences in fatty acid profiles across the spatial and temporal factors (sampling station and season). A cluster analysis was conducted to assess the degree of similarity between FA samples, using the PRIMER statistical package (Clarke and Warwick, 2001). The contribution of individual FAs to similarities and dissimilarities within and between sample groups were tested using similarity percentage analysis routine (SIMPER). Fatty acid ratios were calculated and used as biomarkers based on El- Sabaawi et al. (2009) to inspect if animal, bacteria or algae class ratios were maintained in the lipid extracts of zooplankton species thus reflecting their trophic position and dietary quality. Carnivorous zooplankton show higher quantities of polar lipids (rich in PUFA) than herbivorous crustaceans zooplankton. Thus, the ratio PUFA (sum of all polyunsaturated fatty acids) /SFA (sum of all saturated fatty acids) denote carnivory in copepods (Cripps and Atkinson, 2000). Another index to determine the degree of carnivory was the ratio DHA/EPA (docosahexaenoic acid to eicosapentaenoic acid, 22:6n-3/20:5n-3) (Dalsgaard et al., 2003). DHA is highly conserved in the food webs being an important component of polar lipids (Scott et al., 2002). Thus, the ratio DHA/EPA should increase toward higher trophic levels. Besides the use of DHA/EPA to determine carnivory, this ratio may also reflects the dinoflagellates and diatoms proportions in the diets of omnivorous and herbivorous organisms as DHA is often dominant in dinoflagellates, whereas EPA is mainly found in diatoms (Dalsgaard et al., 2003). The proportion of all diatom markers (D=16PUFA+16:1n-7+20:5n-3) to all flagellate markers (F=18PUFA+18:2n-6+22:6n-3), D/F, was also used to distinguish between diatom and dinoflagellate-based diet (El-Sabaawi et al., 2009). High proportions of 18:2n-6 denote the presence of terrestrial detritus or green algae in zooplankton dietary (Dalsgaard et al., 2003). Because bacteria biosynthesizes large amounts of *iso* and ante-*iso* branched chains containing 15-17 carbons, the sum 15:0+17:0 was used to detect the presence of bacteria in organisms' dietary (Parkes, 1987; Rajendran et al., 1994; Vestal and White, 1989).

RESULTS

Water samples analysis

Water samples were mainly composed of saturated fatty acids (SFA) as C_{16} and C_{18}. SFA were found in highest concentrations (<0.08 mg/ind). C_{18} PUFAs (polyunsaturated fatty acids) were only found in autumn samples of southern arm station (St S2) in very low concentrations (<0.01 mg/ind). Winter samples showed the highest levels of total SFA.

Zooplankton fatty acids composition – general patterns

Species for FA analysis were chosen based on their indicator value (based on densities) within each sampling station. The selected species that were chosen in each station are reported in table 1.

Table 1 – Species sampled in three sampling stations (St M, St S2 and St N2) of the Mondego estuary at winter, spring, summer and autumn of 2010.

	St M	**St S2**	**St N2**
Winter	*Acartia clausi* *Daphnia longispina* *Copidodiaptomus numidicus*	*Acartia tonsa* *Daphnia longispina*	*Daphnia longispina* *Copidodiaptomus numidicus*
Spring	*Acartia clausi* *Centropages typicus*	*Acartia tonsa* *Calanipeda aquae dulcis*	*Daphnia longispina* *Copidodiaptomus numidicus* *Acanthocyclops robustus*
Summer	*Temora longicornis* *Acartia clausi* *Evadne nordmanni* *Podon polyphemoides*	*Acartia tonsa* *Calanipeda aquae dulcis*	*Copidodiaptomus numidicus* *Calanipeda aquae dulcis*
Autumn	*Temora longicornis* *Acartia clausi* *Evadne nordmanni+Penilia avirostris*	*Acartia tonsa* *Daphnia longispina*	*Copidodiaptomus numidicus* *Acartia tonsa* *Eurytemora velox*

Seasonal differences in FA composition are summarized in table 2. Zooplankton species showed higher FA concentrations in winter and spring than in summer and autumn. Indeed, in species that occured all year round in the same sampling station (see Table 3), a sharp increase in FA concentration was observed from autumn to winter. In general, saturated and unsaturated fatty acids were predominant in estuarine and freshwater copepods than in the marine copepod species. The only exception to this finding was *Centropages typicus* since this species showed a similar fatty acid composition as the estuarine and freshwater species with high amounts of HUFA (5.77%). A similar pattern was observed in the cladoceran *Daphnia longispina* showing a higher diversity and concentration of total FAs (SFA = 7.86%; MUFA = 8.25%; PUFA = 2.2% and HUFA = 1.78%), while the marine cladoceran species showed a lower share of fatty acids (<1%) (Table 3). Furthermore, the SFA were mainly composed of mixtures of 14:0; 16:0 and 18:0 and represented about 35% of the total lipid composition. Copepods species showed higher quantities of n-3 PUFA than cladoceran species, whereas *D. longispina* accumulated more ARA (eicosatetraenoic acid, 20:4n-6) (Table 3).

Table 2 –Total fatty acid (FA) concentration (mean ± standard error, in mg/ind) extracted from each species in different seasons. The number below the line indicates the total number of FAs.

		Winter	**Spring**	**Summer**	**Autumn**
Marine species	*A. clausi* (AC)	0.02 ± 0.02 n=12	0.01 ± 0.03 n=5	0.01 ± 0.01 n=7	0.01 ± 0.01 n=3
	E. velox (EV)	-	-	-	0.02 ± 0.01 n=11
	T. longicornis (TL)	-	-	0.01 ± 0.00 n=11	0.01 ± 0.01 n=7
	C. typicus (CT)	-	0.13 ± 0.07 n=13	-	-
	E. nordmanni (EN)	-	-	0.01 ± 0.01 n=10	-
	P. polyphemoides (PP)	-	-	0.01 ± 0.01 n=10	
	E. nordmanni + *P. avirostris* (ENPA)	-	-	-	0.03 ± 0.04 n=6
Estuarine species	*A. tonsa* (AT)	0.04± 0.01 n=21	0.02 ± 0.02 n=9	0.01± 0.01 n=7	0.03 ± 0.04 n=9
	C. aquae dulcis (CAD)	-	0.01± 0.02 n=7	0.01 ± 0.01 n=10	-
Freshwater species	*C. numidicus* (CN)	0.03 ± 0.01 n=35	0.05 ± 0.03 n=11	0.01 ± 0.01 n=8	0.01 ± 0.01 n=6
	A. robustus (AR)	-	0.09 ± 0.04 n=13	-	-
	D. longispina (DL)	0.05± 0.02 n=46	0.04 ± 0.03 n=10	-	0.01 ± 0.01 n=7

Table 3 – Relative fatty acid (FA) concentration (%) in all (A) copepod and (B) cladoceran species at each station.

A		*A. clausi*			*E. velox*			*T. longicornis*			*C. typicus*			*A. tonsa*			*C. aquae dulcis*			*C. numidicus*			*A. robustus*		
	Station	**M**	**S2**	**N2**	**M**	**S2**	**N2**	**M**	**S2**	**N2**	**M**	**S2**	**N2**	**M**	**S2**	**N2**	**M**	**S2**	**N2**	**M**	**S2**	**N2**	**M**	**S2**	**N2**
SFA	14:0	0.34	-	-	-	-	0.06	0.12	-	-	0.81	-	-	-	0.41	0.08	-	0.13	0.03	0.00	-	0.77	-	-	0.22
	15:0	0.14	-	-	-	-	0.01	0.01	-	-	0.10	-	-	-	0.13	0.01	-	0.05	0.01	0.00	-	0.15	-	-	0.12
	16:0	2.13	-	-	-	-	0.54	0.58	-	-	2.59	-	-	-	2.39	1.06	-	1.19	0.19	0.81	-	2.01	-	-	2.23
	17:0	0.06	-	-	-	-	0.02	0.04	-	-	0.09	-	-	-	0.09	0.03	-	0.03	0.01	0.04	-	0.16	-	-	0.10
	18:0	0.63	-	-	-	-	0.32	0.20	-	-	0.69	-	-	-	0.82	1.48	-	0.48	0.05	0.19	-	0.93	-	-	0.70
	20:0	0.01	-	-	-	-	0.00	0.00	-	-	0.04	-	-	-	0.02	0.00	-	0.00	0.00	0.01	-	0.02	-	-	0.05
	22:0	0.01	-	-	-	-	0.00	0.00	-	-	0.00	-	-	-	0.01	0.00	-	0.00	0.00	0.00	-	0.02	-	-	0.00
	24:0	0.00	-	-	-	-	0.00	0.00	-	-	0.00	-	-	-	0.01	0.00	-	0.00	0.00	0.00	-	0.02	-	-	0.00
MUFA	16:1	0.05	-	-	-	-	0.03	0.02	-	-	0.95	-	-	-	0.41	0.00	-	0.20	0.02	0.60	-	1.53	-	-	0.47
	17:1	0.03	-	-	-	-	0.00	0.00	-	-	0.00	-	-	-	0.02	0.00	-	0.00	0.00	0.00	-	0.05	-	-	0.00
	18:1(n- 9)	0.23	-	-	-	-	0.03	0.01	-	-	0.95	-	-	-	0.77	0.00	-	0.18	0.00	0.32	-	1.07	-	-	1.40
	18:1(n- 9)	0.03	-	-	-	-	0.02	0.02	-	-	0.00	-	-	-	0.03	0.00	-	0.00	0.00	0.00	-	0.08	-	-	0.00
	20:1(n- 9)	0.00	-	-	-	-	0.00	0.00	-	-	0.00	-	-	-	0.02	0.00	-	0.00	0.00	0.00	-	0.02	-	-	0.09
	22:1(n-9)	0.00	-	-	-	-	0.00	0.00	-	-	0.00	-	-	-	0.01	0.00	-	0.00	0.00	0.00	-	0.00	-	-	0.00
	24:1(n-6)	0.00	-	-	-	-	0.00	0.00	-	-	0.21	-	-	-	0.02	0.00	-	0.00	0.00	0.00	-	0.04	-	-	0.00
PUFA	18:2(n- 6)	0.00	-	-	-	-	0.00	0.01	-	-	0.00	-	-	-	0.05	0.00	-	0.00	0.00	0.00	-	0.02	-	-	0.00
	18:2(n- 6)	0.02	-	-	-	-	0.01	0.00	-	-	0.40	-	-	-	0.14	0.04	-	0.00	0.00	0.12	-	0.45	-	-	0.62
	18:3(n-3)	0.00	-	-	-	-	0.00	0.00	-	-	0.26	-	-	-	0.18	0.00	-	0.00	0.00	0.12	-	0.69	-	-	1.03
	18:3(n- 6)	0.00	-	-	-	-	0.00	0.00	-	-	0.00	-	-	-	0.01	0.00	-	0.00	0.00	0.01	-	0.03	-	-	0.00
	20:2(n-6)	0.00	-	-	-	-	0.00	0.00	-	-	0.00	-	-	-	0.02	0.00	-	0.00	0.00	0.00	-	0.02	-	-	0.00
	20:3(n- 6)	0.00	-	-	-	-	0.00	0.00	-	-	0.00	-	-	-	0.00	0.00	-	0.00	0.00	0.00	-	0.00	-	-	0.00
HUFA	ARA - 20:4(n-6)	0.00	-	-	-	-	0.00	0.00	-	-	0.00	-	-	-	0.03	0.00	-	0.00	0.00	0.03	-	0.01	-	-	0.00
	EPA - 20:5 (n- 3)	0.06	-	-	-	-	0.08	0.10	-	-	2.14	-	-	-	0.51	0.00	-	0.00	0.00	0.10	-	1.51	-	-	0.48
	DHA - C22:6(n -3)	0.06	-	-	-	-	0.11	0.16	-	-	3.63	-	-	-	0.18	0.00	-	0.00	0.00	0.00	-	0.90	-	-	1.14
	n	**14**	**-**	**-**	**-**	**-**	**11**	**11**	**-**	**-**	**13**	**-**	**-**	**-**	**23**	**6**	**-**	**7**	**6**	**13**	**-**	**22**	**-**	**-**	**13**

Table 3 – [continued]

B		***E. nordmanni***			***P. polyphemoides***			***E. nordmanni + P. avirostris***			***D. longispina***		
	Station	**M**	**S2**	**N2**	**M**	**S2**	**N2**	**M**	**S2**	**N2**	**M**	**S2**	**N2**
SFA	14:0	0.05	-	-	0.04	-	-	0.10	-	-	0.00	0.03	0.14
	15:0	0.01	-	-	0.01	-	-	0.02	-	-	0.00	0.22	0.19
	16:0	0.25	-	-	0.32	-	-	0.75	-	-	1.13	2.46	1.20
	17:0	0.01	-	-	0.01	-	-	0.03	-	-	0.08	0.17	0.14
	18:0	0.08	-	-	0.08	-	-	0.78	-	-	0.50	0.80	0.66
	20:0	0.00	-	-	0.00	-	-	0.00	-	-	0.02	0.00	0.01
	22:0	0.00	-	-	0.00	-	-	0.00	-	-	0.02	0.04	0.00
	24:0	0.00	-	-	0.00	-	-	0.00	-	-	0.02	0.04	0.01
MUFA	16:1	0.03	-	-	0.03	-	-	0.00	-	-	0.29	2.12	1.42
	17:1	0.00	-	-	0.00	-	-	0.00	-	-	0.04	0.00	0.02
	18:1(n- 9)	0.02	-	-	0.03	-	-	0.00	-	-	0.39	0.47	1.71
	18:1(n- 9)	0.03	-	-	0.06	-	-	0.00	-	-	0.10	1.66	0.00
	20:1(n- 9)	0.00	-	-	0.00	-	-	0.00	-	-	0.00	0.03	0.00
	22:1(n-9)	0.00	-	-	0.00	-	-	0.00	-	-	0.00	0.00	0.00
	24:1(n-6)	0.00	-	-	0.00	-	-	0.00	-	-	0.00	0.00	0.00
PUFA	18:2(n- 6)	0.00	-	-	0.00	-	-	0.00	-	-	0.02	0.00	0.00
	18:2(n- 6)	0.00	-	-	0.00	-	-	0.02	-	-	0.06	0.69	0.40
	18:3(n-3)	0.00	-	-	0.00	-	-	0.00	-	-	0.02	0.10	0.67
	18:3(n- 6)	0.00	-	-	0.00	-	-	0.00	-	-	0.00	0.17	0.04
	20:2(n-6)	0.00	-	-	0.00	-	-	0.00	-	-	0.00	0.00	0.01
	20:3(n- 6)	0.00	-	-	0.00	-	-	0.00	-	-	0.00	0.02	0.00
HUFA	ARA - 20:4(n-6)	0.00	-	-	0.00	-	-	0.00	-	-	0.00	0.51	0.16
	EPA - 20:5 (n- 3)	0.11	-	-	0.14	-	-	0.00	-	-	0.01	0.05	0.98
	DHA - C22:6(n -3)	0.07	-	-	0.11	-	-	0.00	-	-	0.00	0.07	0.00
	n	**10**	**-**	**-**	**10**	**-**	**-**	**6**	**-**	**-**	**14**	**18**	**16**

Figure 2 showed that mainly saturated fatty acid components dominated the FA profile of *A. clausi* namely 14:0, 16:0 and 18:0. The SFA 15:0 and 17:0 as indicators of bacterial feeding (Dalsgaard et al., 2003; El- Sabaawi et al., 2009) were found in small amounts (<0.50%) in winter and spring. The monounsaturated fatty acid (MUFA) content was higher in winter than in summer, whereas the highly unsaturated fatty acid DHA recorded the highest value in summer. The fatty acid composition of *Acartia tonsa* was dominated by the same MUFA and SFA as *A. clausi* plus 17:0 (1.47%) in spring. EPA was found in all seasons, except for autumn, whereas the polyunsaturated fatty acid 20:2(n-6) represented less than 1% in spring. The calanoid copepod *C. numidicus* was the indicator species with the highest concentrations of FAs. The SFA and MUFA composition of *C. numidicus* resembled that of *A. tonsa.* The polyunsaturated fatty acids 20:2(n-6), 18:3(n-3), DHA and EPA were found in a specific season, or along the year but showing a large seasonal variation. The huge seasonal variability in FA composition was noticeable for the three indicator species. In winter and spring the species showed the highest richness composition on fatty acids, followed by summer. In autumn the SFA 14:0, 16:0 and 18:0 were the main FAs present in the three species. Indeed, autumn was the season where species revealed the poorest FA composition, with *C. numidicus* showing a slightly higher richness in FA composition than both *Acartia* species. In addition, *C. numidicus* was the species with the highest quantities of FA, mainly MUFA and PUFA, with EPA and DHA being part the top 5 FAs across the year.

Multivariate analysis of the zooplankton assemblages

Cluster analysis (Fig. 3) separated zooplankton species into two groups: Group I included only samples from winter and spring, consisting of species with higher concentrations and diversity of FAs. Group II represented species with lower FA levels and lower FA diversity. This group was subdivided in two smaller groups: group IIA representing summer and autumn samples, whereas in group IIB species from the four seasons were pooled. The species from Group I are grouped based on their higher concentrations of 16:0, 16:1, 18:0, 18:1(n-9), 18:2(n-6) and EPA, while the species of Group II were characterized by higher concentrations of 14:0, 16:0 and 18:0 (see Table 3). The species *A. tonsa*, *C. numidicus* and *D. longispina* presented a high affinity with species of both groups due to the specificity on FA profiles that characterized both groups (I and II). The results of multivariate analysis showed a clear significant difference in the FA composition of zooplankton community, defining two major groups (Fig. 4). The n-MDS plot revealed a clearly seasonal distribution of the sampled species based on FA composition and concentration (stress=0.05). Group A represented the winter and spring samples, whereas in group B all species occurring in summer and autumn were found. ANOSIM analysis indicated a clear separation of the groups defined (R= 0.505; p=0.001).

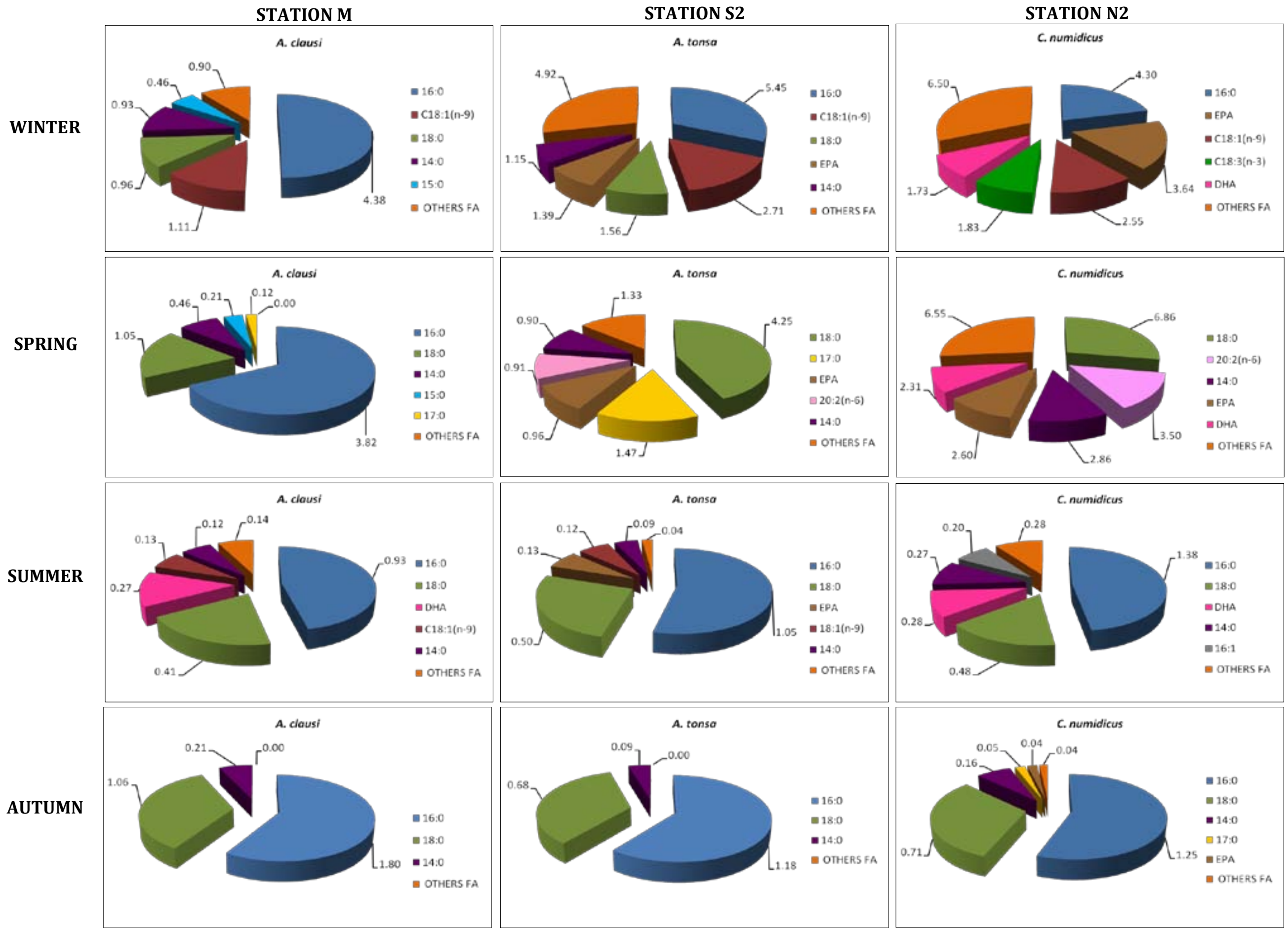

Figure 2 – The 5 most abundant fatty acids in the 3 indicator species (*A. clausi*, *A. tonsa* and *C. numidicus*) of each sampling station (seasonal characterization).

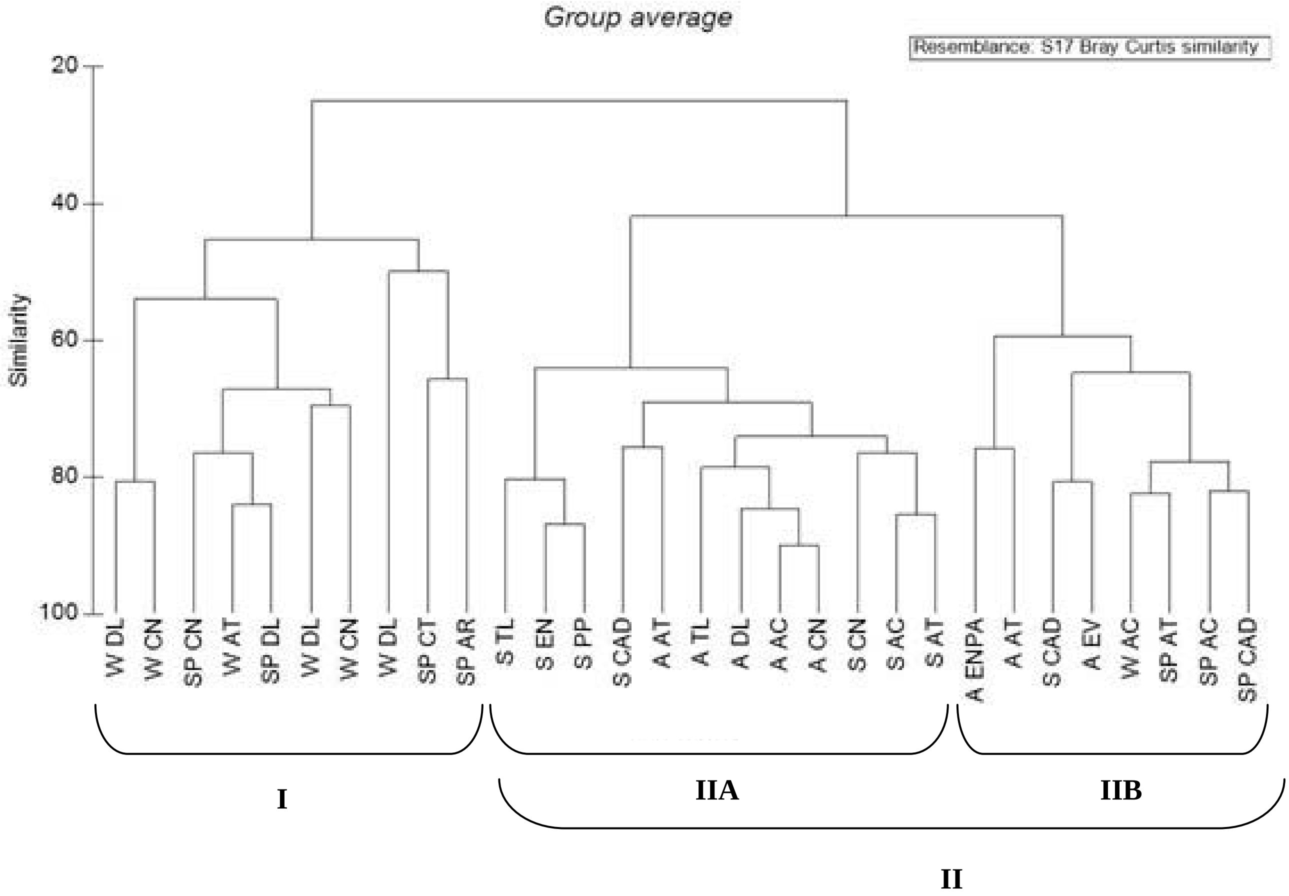

Figure 3 – Cladogram (cluster analysis) grouping zooplankton species based on their total fatty acid composition in different seasons and sampling stations. The two groups (and subgroups) are indicated by roman numbers. W – Winter; SP – Spring; S – Summer and A – Autumn. For species codes see table 2.

Regarding pairwise differences, almost all seasons were significantly different ($p<0.05$) and present a high R-value, indicating a good segregation of groups (winter/summer: R = 0.928; p = 0.001; winter/autumn: R = 0.668; p = 0.002; spring/summer: R = 0.806; p = 0.001; spring/autumn: R = 0.474; p = 0.004). However, non-significant differences were also found between winter-spring (R = -0.023; p = 0.476) and summer-autumn (R = 0.142; p = 0.068). Since some of these species occurred in all seasons, differences between groups appeared to be mainly the result of the variations in its FA composition and concentration. SIMPER analysis showed that in winter/spring 7 FA (by decrease order of importance: 16:0; 18:0; 18:1(n-9); 16:1; EPA; 14:0; 18:2(n-6)) explained 80% of the group similarity, whereas in summer/autumn 3 FA (by decrease order of importance: 16:0, 18:0, 14:0) explained 59.2% of the similarity (Table 4). Furthermore, 72.1% of the dissimilarity between groups was explained by the contribution of the following FA, by decrease order of importance: 16:0; 16:1; 18:1(n-9); 18:0; EPA, DHA; 18:3(n-3); 14:0; 18:2(n-6).

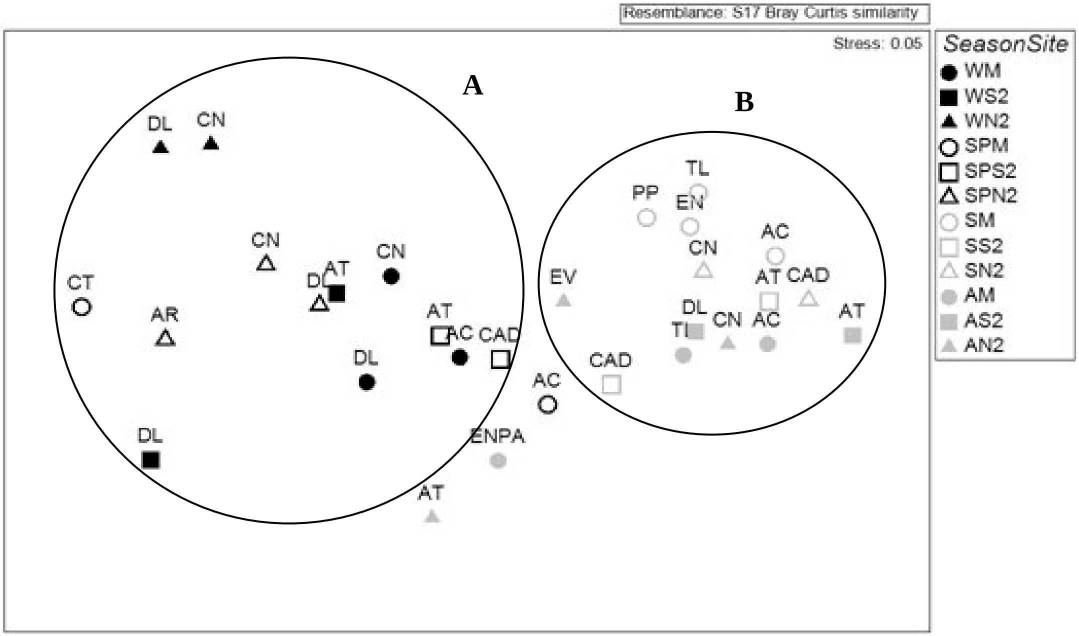

Figure 4 – Two dimensional non-metric MDS ordination plot of fatty acid composition of zooplankton species at the Mondego estuary. Black symbols represent winter and spring sampling species; grey symbols represent summer and autumn sampling species at three stations (mouth station - St M; station of the south arm - St S2 and station of the north arm - St N2) of the estuary. A and B are the different groups defined in the MDS. AC- *Acartia clausi*; AT – *Acartia tonsa*; CT – *Centropages typicus*; TL – *Temora longicornis*; EV – *Eurytemora velox*; AR – *Acanthocyclops robustus*; CN – *Copidodiaptomus numidicus*; CAD – *Calanipeda aquae dulcis*; DL – *Daphnia longispina*; EN – *Evadne nordmanni*; PP – *Podon polyphemoides*; ENPA – *Evadne nordmanni* + *Penilia avirostris*.

Table 4 – Results of SIMPER analysis showing average similarity within the samples groups and average dissimilarity between samples groups, according to MDS analysis.

MDS groups	Similarity	Fatty Acid	Av. % abund.		Av. Sim.	Sim/SD	Contrib. %	Cum. %
W+SP	50.81	16:0	28		19.23	1.30	37.85	37.85
		18:0	9		7.21	2.61	14.19	52.04
		18:1(n-9)	13		7.09	1.62	13.96	66.00
		16:1	14		6.22	1.31	12.24	78.24
		EPA	10		2.61	0.71	5.13	83.37
		14:0	4		1.98	0.85	3.89	87.26
		18:2(n-6)	5		1.57	0.93	3.09	90.35
S+A	59.18	16:0	9		34.78	3.18	58.78	58.78
		18:0	6		14.68	2.26	24.80	83.58
		14:0	1		4.40	2.59	7.44	91.02
	Dissimilarity		**W+SP**	**S+A**	**Av. Diss.**	**Diss/SD**		
W+SP & S+A	72.11	16:0	28	9	18.25	2.04	25.31	25.31
		16:1	14	0	10.07	1.51	13.96	39.28
		18:1(n-9)	13	0	9.95	1.97	13.80	53.08
		18:0	9	6	6.42	1.31	8.91	61.98
		EPA	10	1	6.13	1.08	8.50	70.49
		DHA	10	1	4.72	0.68	6.54	77.03
		18:3(n-3)	5	0	3.54	0.96	4.91	81.94
		14:0	4	1	3.16	1.48	4.38	86.32
		18:2(n-6)	5	0	3.06	1.37	4.25	90.57

Dietary fatty acid biomarkers

In general, the majority of small copepods are omnivorous feeding on small animals and consuming diatoms or flagellates, which is evidenced by the rise of DHA/EPA and the decline or rise of D/F, respectively (Table 5). The dietary quality varies during the year which seems to be dependent on food availability over that period. On the other hand, the ratio DHA/EPA and D/F both pointed at the increased diatom consumption by cladoceran species (Table 5). Still, DHA/EPA displayed an opposite pattern for *D. longispina* in winter at station S2. There were no observable differences in the proportion of bacterial and green algae markers among the different species as their contribution to the diet was in general low.

In terms of spatial variability, at the mouth station, during the warmer months, the decline of D/F reflects the change in dominance from diatoms to flagellates, whereas in winter and autumn an opposite trend was observed. Concerning the station of the southern arm (St S2), copepods showed higher preferences for diatoms in summer and spring months, while flagellates were the first choice in the other months. Based on these biomarkers, *A. tonsa* showed diverse dietary feeding including also small animals. At the station of the north arm (St N2), in winter and spring months, diatoms were the first choice, as well as small animals for copepods species. An exception was observed for *A. robustus* that rather fed on flagellates than on diatoms (Table 5).

DISCUSSION

In the present study, fatty acid profiling allowed to unravel seasonal and spatial changes in functional behavior of zooplankton species from Mondego estuary. Winter and spring were the seasons where species showed the highest concentration and diversity of fatty acids, pointing at the high availability on food quality in the Mondego estuary and the storage behavior of the zooplankton. These high FA concentration and diversity in winter were followed by species collected in spring and summer. Autumn was the season with lowest diversity of fatty acids although species collected in autumn showed a higher concentration of fatty acids than summer. This seasonal pattern in FA profiles is probably largely linked to the seasonal fluxes in seasonal availability of potential food sources.

Table 5 – Seasonal interspecific differences in fatty acid trophic markers (FATMS) for copepods (A) and cladocerans (B) from the Mondego estuary.

A	**Marine Copepod species**							**Estuarine Copepod species**					**Freshwater Copepod species**					
	A. clausi			***E. velox***	***T. longicornis***		***C. typicus***	***A. tonsa***				***C. aquae dulcis***	***C. numidicus***					***A. robustus***
STATION	M			S2	M		M	S2			N2	S2	M	N2				N2
SEASON	W	SP	S	A	S	A	SP	W	SP	S	A	SP	W	W	SP	S	A	SP
DHA/EPA	0.00	0.00	2.17	1.36	2.08	0.29	1.69	0.62	0.00	0.06	0.00	0.00	0.00	0.48	0.66	6.02	0.00	2.39
18:2(n-6)	0.01	0.00	0.00	0.00	0.00	0.00	0.10	0.03	0.01	0.00	0.01	0.00	0.03	0.05	0.06	0.00	0.00	0.15
15:0+17:0[a]	0.03	0.02	0.00	0.01	0.01	0.00	0.04	0.03	0.02	0.00	0.01	0.02	0.01	0.04	0.03	0.00	0.00	0.05
PUFA/SFA[b]	0.04	0.00	0.26	0.20	0.65	0.05	1.49	0.45	0.17	0.08	0.01	0.00	0.36	1.96	0.79	0.15	0.02	0.95
D/F[c]	1.99	0.00	0.46	0.88	0.53	3.50	0.66	0.79	3.44	15.81	0.00	0.00	1.92	1.35	1.03	0.88	0.00	0.28

B	**Marine Cladoceran species**			**Freshwater Cladoceran species**				
	E. nordmanni	***P. polyphemoides***	***E. nordmanni + P. avirostris***	***D. longispina***				
STATION	M	M	M	M	S2		N2	
SEASON	S	S	A	W	W	A	W	SP
DHA/EPA	0.67	0.82	0.00	0.00	2.39	0.00	0.00	0.00
18:2(n-6)	0.00	0.00	0.00	0.02	0.17	0.00	0.05	0.04
15:0+17:0[a]	0.00	0.00	0.01	0.02	0.08	0.02	0.05	0.03
PUFA/SFA[b]	0.47	0.53	0.01	0.07	0.49	0.04	2.78	0.33
D/F[c]	1.86	0.00	0.00	1.63	1.25	0.00	1.77	1.28

Moreover, the FA composition of a particular species varies between seasons but also among stations, within the same season. For instance, *D. longispina* showed different lipid composition from station to station, during the same season, but also among seasons. Furthermore, the south arm station (St S2) is the sampling site where species showed higher concentrations of FAs in winter and spring, while in summer and autumn higher concentrations were found in the marine station at the mouth of the estuary (St M). This variation shows that seasonal feeding by zooplankton species is less clear in the marine part than in other study sites of the estuary in winter and spring suggesting that the prey are less abundant and/or less diverse in FA composition during these seasons. This may explain the relatively low percentage of mono and polyunsaturated fatty acids in marine zooplankton species in contrast to the estuarine and freshwater species. This was also shown when focussing on the 5 most abundant FAs to each indicator species. The marine copepod species *Acartia clausi* was mainly composed by SFA, whereas the estuarine and freshwater species (*A. tonsa* and *C. numidicus*, respectively) present high quantities of MUFA, PUFA and HUFA. Whilst, the higher amounts of n-3 HUFA in copepods species than in the cladoceran species may be explained by the ability of the former group to adjust their n-3HUFA to temperature variations, whereas cladocerans cannot modify their n-3 PUFA content (Farkas, 1979). These variations and low levels of some fatty acids components in zooplankton species is also partly due to the poor fatty acid composition of water samples which highlights the low contribution of water contents to food webs in Mondego estuary.

The present study also allowed revising the food preferences of different planktonic species, highlighting diverse seasonal feeding choices. Copepods typically showed to feed on small animals and diatoms or flagellates species according to food availability, pointing at omnivory behavior. On the other hand, cladoceran species fed mainly on diatom species, showing a herbivorous feeding behavior as efficient filter feeders. Indeed, Bacillariophyceae (diatoms) and Dynophyta (dinoflagellates) are the main groups of phytoplankton in the Mondego estuary, showing the highest abundance in summer and spring (Vieira et al., 2002). Bacteria, diatoms, flagellates and animals are generally reported as part of the diet of copepod and cladoceran species (Arts et al., 2009; Dalsgaard et al., 2003; El- Sabaawi et al., 2009; Samchyshyna, 2008). Thus, zooplankton organisms are typically classified as herbivorous or omnivorous, according to their diet in a particular site and/or season. Therefore, omnivory is reported as a main characteristic for small copepods in temperate regions (Arts et al., 2009; Dalsgaard et al., 2003). Copepods are known to actively select and catch their food particles, being the particle size and the nutritive value important selection factors (Adrian and Scheiner-Olt, 1999; Tackx et al., 1989). According to several authors (Cotonnec et al., 2001; DeMott, 1988) the selection of food particles by copepods, for instance phytoplankton cells, depends on the algal group and the species of grazer and could be defined by a selective index – weakly, intermediate or highly selective - according to the discriminatory behavior. Still, the major fatty acid component of copepod diet is closely related to the selectivity food particle available as the FA profile is a reflection of the consumed food ('you are what you eat' principle). However, one should keep in mind that the selection of a certain food source also depends on the availability of this food source. Therefore, this selective index can largely depend on the study site and time of the year. For instance, Cotonnec et al. (2001) referred *Temora longicornis* to be more selective than *A. clausi*, whereas De Mott (1988) found *T. longicornis* to be weakly selective and *A. clausi* intermediate. Our results suggest a similar selectivity by both species, showing no large difference in FA composition. The calanoid *Calanipeda aquae dulcis* was the only copepod species that did not revealed a significant difference among the trophic markers studied neither a great diversity in fatty acids composition. Indeed, SFA, within the range C_{10}-C_{18} are the dominant fatty acids in this freshwater copepod species' composition, plus few quantities of MUFA, whereas no PUFA was detected. Thus, our findings suggest *C. aquae dulcis* may ingest bacteria, incorporating the FA commonly biosynthesized by bacteria (Dalsgaard et al., 2003). Samchyshyna (2008) reported that despite most of the calanoids being herbivores, filter-feeders or carnivorous behavior, some of them include bacteria and detritus in their diet. Furthermore, in Chesapeake Bay, some copepod species showed to have the ability to ingest suspended detritus and sediments with the associated microfauna (Roman et al., 2001). Small cladocerans have the ability to feed on a wide range of particle types and sizes, showing unselective food preferences (Geller and Müller, 1981; Gophen and Geller, 1984). It is well documented that Cladocera such as *Daphnia* can filter a heterogeneous mixture of bacteria, algae and organic detritus that constitutes an adequate source of food that could vary the relative proportions of the components (Arnold, 1971). The rise of DHA/EPA observed in winter at the station of the south arm to *D. longispina* could be related with a higher amount of organic detritus filtered from the water. Of course, species also make combinations of these different food sources. Still, the different grazing behavior of cladocerans and copepods may have important and contrasting impacts on the FA profile of the mesozooplankton itself as well as of the phytoplankton community, affecting, at last, the zooplankton growth. Indeed, the concentration of essential PUFAs can limit zooplankton growth as they are unable to synthesize these fatty acids at significant rates (Bell and Tocher, 2009; Perumal et al., 2010). Some copepods like *A. clausi* and *T. longicornis* fed on younger stages of copepods, phytoplankton and detritus as reported by Kattner et al. (1981). However, the species ingested by them or other copepods may be composed of different fatty acids composition which may explain the spatio-temporal variations between copepods species.

In addition to species-specific food preferences, McLusky and Elliot (2004) stated that turbidity and tidal currents can limit phytoplankton production and thus food availability for zooplankton species in estuaries. Furthermore, David et al. (2005) found in the Gironde estuary (France), the temporal variations of some copepod species were indirectly controlled by the high suspended particulate matter (SPM) levels, and therefore turbidity. Thus, these two features are potentially responsible for a much reduced primary production and are identified as problematic for copepod's selective feeding. According to previous works (Gonçalves et al., 2010b) there is a close correlation between SPM, associated with other environmental parameters, and zooplankton community structure. Our findings may suggest that these environmental conditions may not be nutritional favorable for some zooplankton species, whereas other species have the ability to prosper and fed within these conditions.

Our work is a first attempt to give an overview of fatty acids composition of zooplankton species present in a temperate shallow estuarine system in southern Europe. Since zooplankton forms an essential link between primary producers and higher order consumers, constituting the source of energy and essential nutrients to the higher trophic levels (Dalsgaard et al., 2003), unraveling the functional role of the first trophic levels will be a pivotal contribution to understand trophic relationships in the Mondego estuary. As lipids are sensitive and good indicators of stress (Arts et al., 2009) and seasonality (present work), they constitute a valuable tool to monitor natural variability in ecosystem functioning. Moreover, they allow to disentangle the effects of this natural variability from the effects of potential threats (e.g. climate change, eutrophication, contaminants or invasive species).

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GENERAL DISCUSSION AND CONCLUSIONS

In this section the various topics studied in the previous chapters will be reported by integrating and discussing the main contribution of this study to a further knowledge of the distribution and composition of (small-sized) zooplankton communities (and their life stages), as well as the fatty acids profiles and dietary preferences of zooplankton species in the Mondego estuary. Outstanding the high sensitivity of zooplankton assemblages to physical and chemical variations that can be manifested in changes in the community structure, zooplankton is considered to be a good indicator of environmental changes (Bonnet and Frid, 2004; Porri et al., 2007). Moreover, during the study period an extreme drought occurred, has been classified by the Portuguese Weather Institute (http://web.meteo.pt) as the worst drought of the past 60 years. Therefore, the study of zooplankton dynamics will be discussed and analyzed concerning the major implications of environmental forcing factors and the extreme weather event.

Small-sized zooplankton assemblages: spatio-temporal distribution and structural patterns

Estuarine systems are unstable habitats characterized by large scale seasonal fluctuations (salinity, temperature, nutrients) and small scale variability (tidal, diel) of the biological and environmental parameters. The distribution and composition of zooplankton species in the Mondego estuary shows a clear association with the environmental gradients, mainly temperature and salinity, observed in the study system. On previous works in the Mondego estuary zooplankton assemblages were collected with 200 μm and 335 μm mesh sized nets (Azeiteiro et al., 1999; Marques et al., 2007, Primo et al., 2009). These larger sized nets conducted an omission or undersampling of the overall contribution of the small-sized zooplankton fraction and the earlier life stages (Azeiteiro et al., 1999; Marques et al., 2007, Primo et al., 2009) that masked the density and biodiversity of zooplankton community. These findings are corroborated by other works (e.g. Hwang et al., 2007; Riccardi, 2010) that document the underestimation of the small-sized fraction of zooplankton by larger sized nets and highlighted the default of these works in terms of the ecological role of this fraction. The previous zooplankton study conducted in Mondego estuary by Vieira et al. (2003) with a 63 μm mesh sized net, only focused the influence of the salinity gradient in the south arm. Thus, the first main conclusion of the present study highlights the limitations of previous works to describe the spatial and temporal distribution of zooplankton assemblages without focusing the zooplankton community as a whole. Indeed, it was evident a clear relationship between environmental factors, mainly a salinity and temperature gradient, with the spatio-temporal patterns of the marine, brackish and freshwater faunal distribution. At the north arm of the estuary higher freshwater species' abundance was found, particularly *Acanthocyclops robustus* and *Copidodiaptomus numidicus* at the upstream areas. However, the south arm was characterized by a brackish community mainly composed by the resident estuarine copepod *Acartia tonsa*. This biological pattern may be explained by the particular hydrological characteristics of the contrasting arms. Thus, at the north arm freshwater river discharges are regularly occurring, whereas the water column of the south arm is more stable due to its lower hydrodynamics shallowness. The marine reaches of the estuary was usually dominated by marine species, mainly the calanoid *Acartia clausi*, *Temora longicornis* and the cyclopoid *Oithona nana* due to the intrusion of marine water. Copepods, mainly nauplii and copepodite stages were clearly dominant along the estuary with the exception of the mouth of the estuarine system due to higher salinities. Furthermore, during rainfall months, nauplii are advected from upstream areas to the mouth of the estuary. Other group quite well represented in the Mondego estuary, but not described yet, is the harpacticoida. A seasonal pattern of dominance was found along the Mondego estuary. *Euterpina acutifrons* and *Paronychocamptus nanus* were the harpacticoid species most abundant, occurring along the study area, with the latter species dominating in winter and spring months. Additonally, adults and copepodites of *Microsetella* sp. and *Paraleptastacus* cfr. *spinicauda* were also dominant in the Mondego estuary. The harpacticoid species identified in the present work showed a peak of density during a specific season along the study area, which allowed grouping them according their distribution and occurrence in the Mondego estuary. Thus, at the upper reaches of the south arm, during winter 2005, a high distribution of *Metis* cfr. *ignea* and *Tachidius discipes* was observed, whereas copepodites of *Tachidius discipes* and *Mesochra* sp. presented a closely affinity due to their high densities in winter 2005 and 2007. During spring 2005/2006, were found the highest densities of *Macrosetella gracilis* and *Parapseudoleptomesochra* sp., while *Microsetella norvegica*, *Nannopus palustris* and *Harpacticus obscurus* showed highest densities in spring 2006. Finally, *Ectinosoma* sp. and *Praeleptomesochra phreatica* showed the highest densities during autumn months of 2006.

The other species identified were considered rare species due to their short occurrence in the Mondego estuary, being representative of one or two sampling sites.

Accordingly to other works (Siokou-Frangou, 1996; Vieira et al., 2003) nauplii of copepod, copepodites and larvae of Polychaeta, Mollusca and Cirripedia showed to be important components of the plankton in the Mondego estuary. Indeed, copepods were the main dominant mesozooplankton group in the Mondego estuary, as stated on other estuarine systems (Kibirige and Perissinotto, 2003; Leandro et al., 2007; Uriarte and Villate, 2005).

Another important conclusion of this study is the clear seasonal pattern showed by copepod assemblages that superimposed an inter-annual variability. The severe drought was responsible for the clear dominance of marine species, which remained along the next regular climatic years. A similar trend was claimed on previous studies of zooplankton (Primo et al., 2009) and of fish (Martinho et al., 2007). Regarding seasonal variations, winter is characterized mainly by freshwater species and lower densities of nauplii, whereas spring and summer are dominated by marine and estuarine species, mainly juveniles. Indeed, in winter due to a higher river flow, waters are less saline and present a high concentration of nutrients from the fields. On the other hand, summer is characterized by saline and warmer water. Furthermore, the distribution and vertical patterns of copepods cannot be analyzed taking into account only the species' response to a gradient of environmental parameters (e.g. temperature and salinity). It is also needed to examine their vertical and horizontal behaviors in terms of a dynamic complex response to tidal, diel and lunar cycles associated with environmental factors and reproductive cycles of predators and preys (Forward Jr. and Tankersley, 2001). Indeed, in the present study other interesting conclusion was that depth and tidal cycles were the main physical conditions responsible for vertical migrations accomplished by horizontal movements or retention of copepod species. Furthermore, a clear dependence between the structure of copepod community and the lunar cycle was observed, with almost all copepod species occurring at high densities in neap tide, with exception of the estuarine species that presented the highest densities in spring tide. Indeed, the resident estuarine species and copepodites stages showed a clear distribution mainly at the bottom during ebb tides, avoiding leaving the estuary. An opposite behavior was observed on *O. plumifera* and nauplii that presented higher densities at surface flood tides, being carried out to coastal areas. These findings are corroborated by Kennish (1990) that reported the structure of copepod community along the estuary depends of both neap-spring and ebb-flood cycles. A similar trend was reported by Titelman and Fiksen (2004), considering that nauplii and small species are less sensitivity to visual predators which explains their vertical behavior. At the estuarine area, neritic species distributed mainly at downstream stations and at the mouth of the estuary, preferring saline waters, whereas resident species distributed mainly at the upper areas of the estuarine system. In this way, earlier stages were transported to downstream stations and to the mouth of the estuary, while adults returned to upstream areas, the area of adult populations. At this study, a novelty about the behavior of *O. nana* and copepodites stages, not yet explored, was described. *O. nana* revealed a resident estuarine behavior, reproducing and performing the development stages inside the Mondego estuary. Copepodites of *O. nana* revealed a wide distribution along the estuary, mainly at downstream stations, whereas at the upper reaches of the estuary were found higher densities of the adults. A similar pattern was also found in Southampton Water estuary (Williams and Muxagata, 2006). Indeed, this species and its earlier stages show a sharp spatial distribution worldwide related to biochemical characteristics (Porri et al., 2007; Williams and Muxagata, 2006). Concerning *O. plumifera,* this species revealed to be one of the dominant species in the Mondego estuary which evidence that this cyclopoid has been underestimated at previous works (Azeiteiro et al., 2000; Marques et al., 2007; Primo et al., 2009). In fact, communities must be study as a whole for an adequate estimate of fluxes, rates and ecological processes (Antacli et al., 2010; Riccardi, 2011).

Fatty acids composition of zooplankton species

The present study reveals the first data of fatty acids (FAs) composition and dietary preferences of zooplankton species in the Mondego estuary. A clear seasonal pattern in composition and abundance of fatty acids were observed. Winter and spring zooplankton species presented the maximal concentrations and diversity of total fatty acids, with the estuarine and freshwater species presenting a higher content of saturated and unsaturated fatty acids rather than the marine species. The marine calanoid *Centropages typicus* was an exception, presenting a similar FA profile as estuarine and freshwater species. According to the principle "you are what you eat", FA profiles reflect the food consumed by each organism. Copepods are known to actively select and catch their food particles, whereas cladocerans do not show selective food preferences (Adrian and Scheiner-Olt, 1999; Cotonnec et al., 2001; Gophen and Geller, 1984). Furthermore, omnivory is reported as a main characteristic of the great part of small copepods from temperate regions (Arts et al., 2009; Dalsgaard et al., 2003). Bacteria, diatoms, flagellates and small animals are all part of copepod and cladoceran diet preferences. In general, copepods fed on algae or small copepods, revealing an omnivores behavior. The exception was the calanoid *Calanipeda aquae dulcis* revealing feeding preferences on bacteria. These finding are corroborated by other studies (Roman et al., 2001; Samchyshyna, 2008) that reported that some calanoid species include on their diets bacteria and detritus. Considering the cladoceran species, these small crustaceans revealed a herbivore behavior being efficient filter feeders. In literature it is well documented the wide range of particle types and sizes filtered by small cladocerans (Arnold, 1971; Gophen and Geller, 1984). The seasonal pattern on dietary preferences, and thus on FAs profiles, of zooplankton species may suggest seasonal fluxes in food availability and thus on prey abundance on each season due to environmental variations at the estuarine area. Indeed, McLusky and Elliot (2004) reported that the quantity of food availability for the zooplankton species is a consequence of forcing factors, such as turbidity and tidal currents, which may limit the phytoplankton production. In addition, David et al. (2005) reported that high suspended particulate matter, and thus turbidity, are the main responsible features for the reduction on primary production. Despite the knowledge on zooplankton community structure associated to environmental factors, a further knowledge on phytoplankton community in the Mondego estuary is undoubtedly important to evaluate trophic relationships.

CONCLUSIONS

The present study integrated and synthesized important information about the distribution and trophic ecology of small-sized zooplankton community in a heterogenous and dynamic estuarine system, the Mondego estuary. It was clearly reported the response of zooplanktonic species (including their life stages) to physical and chemical environmental factors as well the relationship between these variables and the biological component. These communities are very sensitive to environmental variations, occurring drastic changes on their structure in response to severe weather events, with crucial impacts on the trophic food web and thus in the ecosystem. Zooplankton plays an important position on the trophic food web, being the main link in matter and energy between primary producers and higher trophic levels. Thus, the present work also contributed to the characterization on fatty acids composition and the trophic position that zooplankton species occupy on the food web. The feeding behavior of estuarine and freshwater species gives them higher concentration and diversity of fatty acids than the marine species' behavior. In terms of the two main groups studied, copepods feed on algae (e.g. diatoms, flagellates), small copepods and bacteria, whereas cladoceran species present a typical herbivore behavior. These dietary preferences are closely linked to the food availability in the system, which is in turn related with physical, chemical and biological components of the ecosystem. An abrupt environmental change in the ecosystem is followed by changes in the communities structure and thus on the feeding behavior. Indeed, chemical, physical and biological dynamic components are extremely correlated among them and very dependent of unexpected climate change that occurs frequently throughtout the world. Thus, aquatic ecosystems, mainly estuaries, reach great fluctuations which results in continuously changes and adaptations of communities to environmental factors.

FUTURE PERSPECTIVES

During the scientific work of this thesis, new challenges and ideas arouse providing potential research lines that are summarized below.

Long time-series studies

The biological time series representative of the whole zooplankton community should continue being conducted at the Mondego estuary, allowing evaluating the impact of climate variability and extreme events on the zooplankton fauna in a far extension. Furthermore, time-series also acts as monitoring programmes to identify future impacts (e.g. pollutants) that threat the estuarine system. It is undoubtedly important the study of long time-series to determine and understand the relationships between environmental factors and biota. Future research linking plankton time-series of specific geographical areas should be conducted in order to compare and evaluate plankton responses to a variety of scale events worldwide.

Climatic changes and secondary production

At the present study were identified trophic relationships between primary producers-primary consumers. However, further research on trophic relationships must be conducted, mainly between secondary consumers and on predatory interactions in order to understand the dynamics of trophic food webs. Furthermore, the Earth's climate is gradually warming and a number of ecological changes are already being observed. For instance, the distribution of gelatinous zooplankton populations has increased in the recent years in many regions worldwide, linked to physical processes and environmental gradients in the aquatic systems. Jellyfish respond quickly to environmental changes by increasing their feed, reproduction and growth. Therefore, this group have been suggested as key indicator of changing climate conditions (Richardson et al., 2009). Furthermore the zooplankton group has a large impact on lower trophic levels and a great influence on animals with great interest to humans (e.g. fish) (Brodeur et al., 2011). Indeed, some fish species are specialised and highly dependent to feed on most gelatinous zooplankton. Therefore, the study of this research line should be conducted in order to include the gelatinous animals on the study, contributing for the paucity of information data on trophic food web and predatory interactions in the Mondego estuary.

Spatio-temporal distribution and composition of phytoplankton in the Mondego estuary

The study of the phytoplankton assemblages is fundamental, since these organisms usually constitute one of the main source of matter and energy of the food webs in the most productive aquatic systems, namely in estuaries. In general, temperate estuaries are subjected to high variability, depending mainly on freshwater inflow and tides (high-low tides, neap-spring tidal conditions). In many cases these processes drastically modify the estuarine *habitat* properties, which lead to pronounced fluctuations in the composition and dynamics of natural phytoplankton communities. Till this moment there are not any studies on phytoplankton ecology in the Mondego river estuary. Knowledge of the structure of the plankton community and the autoecology of individual *taxa* is a unique source of information in the study of the phytoplankton dynamics in estuarine environments. The knowledge of the abundance, composition and distribution of these organisms along the estuarine gradients provide valuable information on the strength of the physical environment and the structure of the trophic webs. In addition should be started the basis for an inter-annual variability study in order to understand the effects of stochastic and short-term scale events in estuarine communities. A third issue should be to determine the contribution of this community to the food web. Thus, a characterization of the fatty acid composition of phytoplankton species should be conducted to complement the present study.

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