



DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

Mesozooplankton biomass and secondary production in a temperate estuary: effects of processes operating at different time scales

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Professor Doutor Miguel Ângelo Pardal (Universidade de Coimbra) e da Doutora Sónia Cotrim Marques (Investigadora pós-doc do CFE, Universidade de Coimbra)

Dina Amanda Mendes

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RESUMO

A variabilidade estuarina reflecte-se na dinâmica das populações biológicas, particularmente as planctónicas. Um passo fundamental na caracterização das comunidades planctónicas marinhas é o conhecimento da variabilidade da sua biomassa e produção, de forma a avaliar o fluxo de matéria através da rede alimentar e poder estimar o destino desta energia. A biomassa e produção secundária do zooplâncton, com ênfase na comunidade dos copépodes, foram investigados numa estação fixa localizada na boca do estuário do Mondego (Portugal) ao longo do período de um ano, para cada estação. De forma a estudar a influência dos processos físicos a diferentes escalas temporais, foram recolhidas amostras a cada hora, próximo do fundo, e na sub-superfície em ciclos diários e de maré, durante as marés mortas. Os copépodes dominaram a biomassa durante a primavera ($0.585 \pm 0.671 \text{ mg C m}^{-3}$), verão ($0.287 \pm 0.383 \text{ mg C m}^{-3}$), e inverno ($0.221 \pm 0.128 \text{ mg C m}^{-3}$). Os valores mais baixos de biomassa observados deveram-se provavelmente à maior dimensão da malha utilizada em comparação com outros estudos prévios em sistemas estuarinos. Para a biomassa total do zooplâncton, nenhuma variação significativa ocorreu entre estações. No que diz respeito ao ciclo diário, ocorreu variação significativa durante a primavera e o inverno, com valores de biomassa substancialmente mais altos a serem registados para a superfície e fundo. Além disso, a biomassa dos copépodes foi consistentemente maior no fundo durante o inverno. Nesta estação, as amostras do fundo apresentavam biomassa de copépodes mais elevada, quer nas enchentes como nas vazantes. Das análises GLM realizadas para cada estação, a clorofila a , o ciclo diário, e a profundidade foram factores significativos. Ao longo da primavera e inverno, a produção secundária variou de acordo com o ciclo diário e a profundidade. A partir disto, é possível concluir que os factores locais (fluxo do rio, predação, limitação alimentar) regulam as variações sazonais da biomassa e produção secundária do zooplâncton no estuário do Mondego.

Palavras-chave: Estuário do Mondego; Copépodes estuarinos; Biomassa e produção secundária; Variações sazonais; Migrações verticais diárias

ABSTRACT

Estuarine variability is reflected on the dynamics of biological populations, particularly planktonic ones. A fundamental step in characterizing marine planktonic communities is the knowledge of the variability of their biomass and production, in order to assess the flux of matter through the food web and estimate the fate of this energy. Zooplankton biomass and secondary production with emphasis on the copepod community were investigated at a fixed station located at the mouth of Mondego estuary (Portugal) over a one-year period, for each season. In order to study the influence of physical processes at different temporal scales, hourly samples were collected near the bottom and at sub-surface, in diel and tidal cycles, over neap tides. Copepods dominated the biomass during spring ($0.585 \pm 0.671 \text{ mg C m}^{-3}$), summer ($0.287 \pm 0.383 \text{ mg C m}^{-3}$), and winter ($0.221 \pm 0.128 \text{ mg C m}^{-3}$). Lower observed biomass values were probably due to the higher mesh size used in comparison to other previous studies in estuarine systems. For total zooplankton biomass, no differences were detected between seasons. Concerning diel cycle, significant variation occurred mainly in spring and winter, with substantially higher biomass values being registered at night for both the surface and bottom. Moreover, copepod biomass was consistently higher at the bottom during winter. In this season, bottom samples presented higher copepod biomass both at ebb and flood tides. From the GLM analyses run for each season, chlorophyll *a*, diel and depth were significant factors. Along spring and winter, secondary production varied according to diel phase and depth. In winter, night and bottom samples revealed consistently higher daily secondary production rates. From this, it is possible to conclude that local factors (river flow, predation, food limitation) regulate the seasonal variations in zooplankton biomass and production in the Mondego estuary.

Keywords: Mondego estuary; Estuarine copepods; Biomass and secondary production; Seasonal variations; Diel vertical migrations

Chapter 1

INTRODUCTION

1.1 Estuarine and coastal habitats of plankton

Estuaries are transitional zones between saltwater and freshwater ecosystems, and have always been in close association with human populations (Nybakken, 2001). In temperate zones, they support large fisheries, aquaculture, tourism and recreational activities, as well as intense agriculture (Gilbert, 2001). Despite exploitation and destruction by man, the estuarine habitat remains as one of the most resilient and productive on earth, providing unique ecosystem services, such as: the trapping of contaminants in sediments, provision of nursery grounds for marine fish, and feeding areas for migratory birds (McLusky & Elliott, 2004). They also play an essential part in processes of decomposition, nutrient cycling, and flux regulation of water (Kennish, 2002).

The dynamic nature of estuarine ecosystems is defined by varying physical and chemical characteristics, including fluctuations in salinity, temperature, turbidity, and oxygen in the water column. Substrate composition and the action of waves and currents are also important. Water circulation and land influence induce high variability on the distribution and structure of planktonic populations, which tend to be spatially and temporally heterogeneous when compared to other aquatic systems (Marques *et al.*, 2007). Although these habitats are considered stressful and harsh, the few organisms that are able to survive and adapt to them manage to escape from competition in the sea or surrounding freshwater, and often thrive in large numbers. In fact, all estuaries show a gradual reduction in diversity, but not in abundance or productivity of species when compared to adjacent aquatic habitats (Elliott & McLusky, 2002). According to Wildish (1977), community composition, biomass and productivity are controlled by three major factors: food supply, supply of colonizing larvae, and interspecific competition. Capability of using the rich food supply available becomes the main factor controlling biomass and productivity of estuarine fauna.

1.2 Plankton food webs

The term 'plankton' commonly refers to all organisms that drift in water, whose abilities of locomotion are insufficient to withstand currents.

Zooplankton is the animal fraction of this small universe, and together with phytoplankton and bacterioplankton, constitutes the plankton community of estuaries and coastal zones (Day *et al.*, 1989). Plankton food webs are complex and dynamic because of the short term, seasonal, and annual variations in species composition and trophic relationships within the water column (Johnson & Allen, 2005). In warm temperate coastal systems such as the Mondego estuary, planktonic food webs tend to be based on detritus, which is derived from local macrophytes and material imported into the estuary. This detritus is the main diet of deposit and suspension feeders, while some of the original plant material is eaten by herbivores. Detritivores are then consumed by vertebrate and invertebrate predators such as fish, birds and crabs. Parallel to this detritus-based chain is a closely interacting phytoplankton-based chain (Little, 2000). In most coastal ecosystems, phytoplankton dominate ecosystem primary production (Cloern, 2001; Harding *et al.*, 2002), and its cycles are intimately linked with zooplankton dynamics.

Due to their significant abundance and biomass in estuarine and nearshore areas, zooplankton play a key role as major grazers in aquatic food webs, being critical intermediaries in the flow of energy and matter through marine food chains, from primary producers to consumers at higher trophic levels, such as fish, marine mammals, and turtles (Richardson, 2008).

Zooplankton are extremely diverse structurally and taxonomically, consisting of many larval and adult forms that represent most of the phyla of the animal kingdom (Ré *et al.*, 2005; Suthers *et al.*, 2008; Marques *et al.*, 2009 a). Meroplanktonic communities, that include temporary residents of the plankton (e.g. juveniles, larvae and eggs of adult forms from the benthos or nekton), tend to exhibit more taxonomic diversity than the holoplankton (permanent plankton), which is dominated by large populations of crustaceans, especially copepods (Omori & Ikeda, 1984; Feinberg & Dam, 1998; Marques *et al.*, 2009 a).

In most estuarine ecosystems, copepods comprise the bulk of the holoplankton (Day *et al.*, 1989), even outnumbering insects by possibly three orders of magnitude. They include species from the *Acartia*, *Eurytemora* and *Pseudodiaptomus* genera (McLusky & Elliot, 2004). The relevance of copepods is such, that the reproductive and recruitment success of several pelagic fish and shellfish species of high economic value

are extremely dependent on the dynamics of their populations (Conover *et al.* 1995; Beaugrand *et al.* 2003).

1.3 Spatio-temporal variation and diel vertical migrations

Over time, zooplankton does not reveal a uniform composition in the water column for different regions and bathymetries. In spite of this, variations in biomass and structure may be similar in successive years, enabling annual and inter-annual analysis of community composition and succession of populations (Parsons & Takahashi, 1973; Bougis, 1974; Omori & Ikeda, 1984). Geographic ranges of drifting organisms are primarily determined by water temperatures, which are a function of latitude and ocean currents. Within a given temperature range, salinity is the most influential distribution factor (Marques *et al.*, 2006). Numerous species show preferences for specific habitats or hydrographic conditions with different salinity levels. Differences in depth, current velocity, tidal action, and turbidity affect local distribution (Johnson & Allen, 2005).

The composition and abundance of oceanic, neritic and estuarine zooplankton are distinct and display a non-homogeneous, variable spatial distribution (Ré *et al.*, 2005). Neritic and oceanic distribution can be recognized by differing characteristics (Bougis, 1974). In the first case, certain species are abundant in coastal zones where physico-chemical water factors fluctuate considerably. In the second, species area distribution is characterized by water with greater physico-chemical stability, mainly regarding temperature and salinity. While neritic plankton is composed of numerous meroplanktonic larvae of benthic organisms, oceanic plankton contains mostly larval forms of nektonic animals. Some species of zooplankton display an intermediate distribution between coastal and oceanic zones (e.g. Chaetognaths of the genus *Sagitta*) (Ré *et al.*, 2005).

Fluctuations of environmental factors are more significant and complex in estuarine systems due to the combination of land and ocean influences (Sikou-Frangou, 1996). Zooplankton distribution is influenced by factors acting on many scales, with multiple interactions in an unstable aquatic environment. For instance, estuaries are strong advective environments as a direct consequence of the tidal currents (ebb and

flood) and river flow, imposing an additional stress factor for zooplankton populations (McLusky & Elliot, 2004). Along an estuary there is usually a sequence of planktonic assemblages transitioning from freshwater (oligohaline) species, to estuarine, and finally marine (stenohaline) species (Little, 2000).

Zooplankton exhibit a variety of daily cycles including vertical and horizontal migrations, changes in feeding behaviour, and alternative reproductive states (Haney, 1988). Although exposed to forces of turbulence, almost all species have developed some way of movement, even if only to change their vertical position in the water column (Lenz, 2000). Many of these creatures undergo active vertical migrations with a circadian rhythm (Raymond, 1983), known as diel vertical migrations (DVMs). These occur in a wide range of zooplankton taxa and probably represent the largest synchronized animal migration in terms of biomass on the planet (Hays, 2003). The major stimulus that controls these movements is light, with vertical migrators responding negatively by sinking down during the day and rising at night, which allows them to take advantage of inflowing and outflowing currents at various depths within the estuary. This is the most common pattern, but reverse diurnal migrations have also been documented (Nybakken, 2001).

The adaptive advantage of these movements is not completely understood, but common explanations include the avoidance of predators, and damaging solar radiation (Haney, 1988). Moreover, estuarine zooplankton are constantly faced with the risk of being washed out to sea, but manage to remain in the estuary by persisting in the layer between the surface brackish water and salt wedge (halocline), or near the vegetation along the bottom and sides of the estuary (Redden *et al.*, 2008). Species alternate swimming patterns according to the ebb and flood tides, and passive movements upstream of particular size fractions have also been demonstrated (Johnson & Allen, 2005). This suggests that there is an important link between hydrological cycles and zooplankton community ecology in these habitats. It has been proposed that migrating zooplankton can cause an active transport of carbon from ocean surface layers, because of the amplitude of DVM (up to hundreds of metres) with the pattern of deep daytime fasting and shallower nighttime feeding (Hays *et al.*, 2001). Even though the Mondego estuary is a shallow system, Marques *et al.* (2009) have confirmed that zoo-

plankton abundance attains higher densities at night than during the day, supposing a flux of biomass that is in agreement with this idea.

Meroplanktonic forms of various taxonomic groups (in particular larvae of Hydrozoa, Polychaeta, Crustacea, Decapoda, Echinodermata and ichthyoplankton) may dominate neritic and estuarine plankton during certain periods of the year (Ré *et al.*, 2005), as evidenced in the Mondego estuary (Marques *et al.*, 2006, 2007; Morgado *et al.*, 2007). Holoplankton go through equally significant variations over annual periods. Copepods tend to dominate collected samples, and species succession with maximum being peaks of occurrence often visible. In temperate systems, other zooplankton groups are also well represented (Appendicularia, Cladocera, Decapoda, Chaetognatha, Mysidacea, Cnidaria, among others), and maximum abundances are usually noticeable in spring and autumn.

This patchiness and constant change in distribution in time and space in the pelagic realm often makes the study of plankton difficult – each sample collected is unique and replicates do not exist.

1.4 Biomass and secondary production

Productivity has become a central and extensively studied aspect in marine plankton research over the last few decades (Runge & Roff, 2000). It is of interest as a measure of energy flow through a population and as an indicator of its physiological or nutritional state (Kimmerer, 1987).

Secondary production is defined as the biomass produced by a population in a time interval, regardless of whether it survives to the end of that interval (Clarke, 1946; Ricker, 1946). Production is estimated from animal biomass measurements, and from growth and mortality rates of the population analysed (Lopes, 2007). Measurements of secondary production are necessary to provide flux estimates crucial to the understanding of ecosystem function. Biomass can be expressed as dry mass (mg m^{-3}), and is equivalent to the amount of living substance constituting the organisms under study. The simplest means of quantification is to identify, count and weigh the organisms within particular parts of the estuary (Little, 2000).

Although biomass measurement is straightforward, the same cannot be said for growth and mortality in cohorts over different time intervals (Lopes, 2007; Kimmerer 1987). Traditional techniques developed to measure these rates, such as cohort analysis and cumulative growth studies (Bougis, 1974; Rigler & Downing, 1984) are based on the collection of data at short sampling intervals along one or more generations. This is both time consuming and labour intensive, making it impractical to make comprehensive measures over large areas with high spatial and temporal resolution (Hirst & Bunker, 2003). A number of globally applicable models have been developed to predict secondary production from a few more easily measured parameters, like temperature (Huntley & Lopez, 1992), or temperature and body weight (Ikeda & Motoda, 1978; Hirst & Lampitt, 1998). However, these methods are limited and often overestimate zooplankton growth. A more recent model suggests that chlorophyll *a* concentration is a good alternative to predict copepod weight-specific fecundity and growth rates (Hirst & Bunker, 2003).

Copepods comprise up to 80% of the mesozooplankton biomass (Kiørboe, 1998), and are the most significant component of marine and estuarine environments as herbivores and prey for higher levels (e.g. fish larvae and crustaceans) (Leandro *et al.*, 2007; Miyashita *et al.*, 2009). Regulation of nutrients occurs through their excretory activities (Ikeda & Motoda, 1978), and they also contribute to the downward flux of organic material (Feinberg & Dam, 1998). They play a fundamental role in exporting, redistributing, and repackaging carbon and nutrients in the upper ocean (Banse, 1995). The copepod community of the Mondego estuary is dominated by the *Acartia* genus (Azeiteiro, 1999; Vieira, 2003 a). These organisms reproduce throughout the year, and several annual growth generations can be found in temperate systems like this one because of favourable temperatures and high food availability (Klein & Gonzalez, 1988; Kleppel, 1992).

Secondary production of copepods is of two types: growth production, and egg production. Several methods have been developed to measure copepod growth rates (see Runge & Roff, 2000 for a review). Besides those mentioned above for zooplankton, weight-specific egg production (Poulet *et al.*, 1995; Hirst & McKinnon, 2001) and physiological models (Ikeda *et al.*, 2001) can also be used to determine copepod

growth rates from preserved samples. Even though egg production methods estimate only part of the production of copepods, they should be adopted as a standard for comparing different habitats worldwide (Poulet *et al.*, 1995).

1.5 The Mondego estuary: study case

The Mondego estuary is located on the west coast of Portugal facing the Atlantic (40°08' N, 8°50' W), and has a warm temperate climate. With an area of about 3.3 km², it comprises two arms, north and south, that are separated by the small Murraceira Island (Fig. 1). The two branches have different hydrographic characteristics and separate upstream at 7 km from the sea, joining again near the mouth. The north arm is deeper (4 to 8 m during high tide, tidal range of 1 to 3 m) and presents low residence times (<1 day). It constitutes the main navigation channel of the Figueira da Foz harbour and suffers from regular dredging activity. The southern arm is shallower (2 to 4 m during high tide, tidal range of 1 to 3 m) and is almost silted up in the upstream areas, causing freshwater of the river to flow essentially through the north arm. Water circulation in this arm is mostly due to the tides and a small input of freshwater from the Pranto River tributary, which is controlled by a sluice located 3 km from confluence with the Mondego River. Due to the differences in depth, tide penetration is faster in the north arm and causes greater daily changes in salinity, whereas temperature shows more daily variations in the south arm (Marques *et al.*, 2002).

The physical and chemical dynamics and the ecology of shallow mesotidal estuaries like the Mondego are largely influenced by freshwater runoff and hydrological exchange with the open sea. Freshwater input creates salinity gradients and stratification, assuring a large transport of silt, organic material and inorganic nutrients. Open marine areas determine large scale physical and chemical forcing on the ecosystem by the action of wind and tides (Berner & Berner, 1996). These characteristics ensure efficient water column mixing inside the estuary, and re-suspension with fast vertical transport of organic and inorganic matter that integrate the pelagic and benthic food webs (Duarte *et al.*, 2002). During neap tides in the northern arm of the Mondego estuary, a salt-wedge is formed at low tide, and changes to a partially mixed water

column at high tide. At spring tides a contrast occurs, with partial mixing at low tide and considerable mixing at high tide. Water in the south arm is usually completely mixed, but may be subject to partial mixing and show some stratification during fluvial floods, which are rare (Cunha & Dinis, 2002).

In general, environmental conditions in the Mondego estuary provide a large variety of aquatic habitats for diverse populations of marine, brackish and freshwater species of fauna and flora. Salinity and water temperature gradients in particular are favourable for the establishment of abundant plankton communities (Marques *et al.*, 2009 a). This system has been extensively studied for over two decades on various levels that have provided a large database of information regarding its functioning, community structure and diversity, nutrient cycling and plant dynamics, existing environmental threats, and the development of tools for environmental monitoring, assessment, and management (Marques *et al.*, 2002).

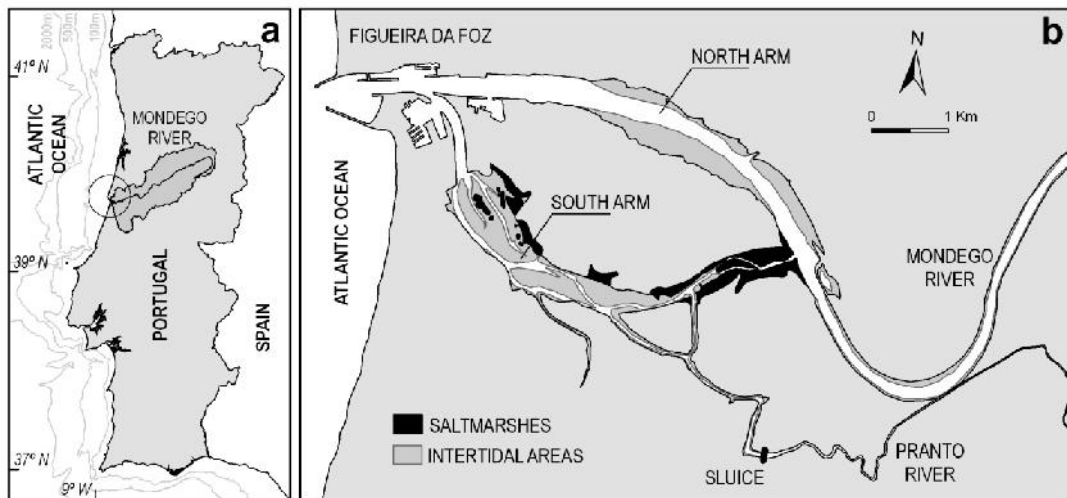


Figure 1. Location of the Mondego estuary on the west coast of Portugal (a); detail of the estuary showing the location of salt marshes and intertidal areas (b).

1.6 General objectives

In Portugal, marine zooplankton studies have focused on several topics, including: biomass and abundance (Sprung, 1994; Villa *et al.*, 1997; Morgado *et al.*, 2003; Leandro *et al.*, 2007), metal stress (Monteiro *et al.*, 1995), contamination and pollution (Monterroso *et al.*, 2003; Pereira *et al.*, 2007; Cardoso *et al.*, 2013; Frias *et al.*, 2014),

composition and distribution (Caldeira *et al.*, 2001; Queiroga *et al.*, 2005; Chícharo *et al.*, 2006; Mendes *et al.*, 2011), and environmental forcing (Sobrinho-Gonçalves *et al.*, 2013). The zooplankton communities of the Mondego have been extensively characterized and studied on various levels by a number of researchers (Vieira *et al.*, 2003 a, b; Pastorinho *et al.*, 2003; Marques *et al.*, 2006, 2007, 2009 b, 2014; Morgado *et al.*, 2006; Primo *et al.*, 2009), but none have focused on the exchange of biomass and secondary production with the estuary and coastal waters.

In order to better comprehend the role of zooplankton in the material flow of this ecosystem, the principle aims of this study were:

1. to quantify zooplankton biomass categorized by taxonomic groups;
2. to estimate secondary production with emphasis on the copepod community;
3. to evaluate the effect of processes operating at different time-scales on biomass transport at the interface between the estuary and the adjacent coast: seasons, tidal and diel cycles.

Chapter 2

MATERIALS AND METHODS

2.1 Sampling programme and laboratory procedures

Zooplankton samples were collected at a fixed station located at the mouth of the Mondego estuary (Fig. 2.) over the course of a year, from summer 2005 to spring 2006. The sampling station was characterized by depths of 6 to 13 m. In this area, the influence of both river flow and coastal waters is strong. Seasonal sampling took place in June, September, October, and December of 2005, March, and April of 2006, during neap tides. Collection was performed hourly over diel cycles (25 h), from two depth ranges: sub-surface and 1 metre above the bottom. Samples were classified as day or night, with the day phase being taken from sunrise to sunset, and the night phase from the corresponding period. Zooplankton was gathered by horizontal tows at low speed, using a bongo net of 335 μm mesh size (0.5 m mouth diameter, 3 min tow, 2 knots) equipped with a Hydro-Bios flowmeter to calculate the filtered volume (average 20 m^3). Samples were fixed and preserved in a 4% borax-buffered formaldehyde seawater solution. In parallel with the tows, water temperature (T), salinity (S), dissolved oxygen (DO) and pH were recorded at each collection point with an appropriate sensor at both depths. Turbidity was measured using a Secchi disk. Water samples of 500–1000 ml were also filtered for determination of chlorophyll *a* (Chl *a*) and suspended particulate matter (SPM).

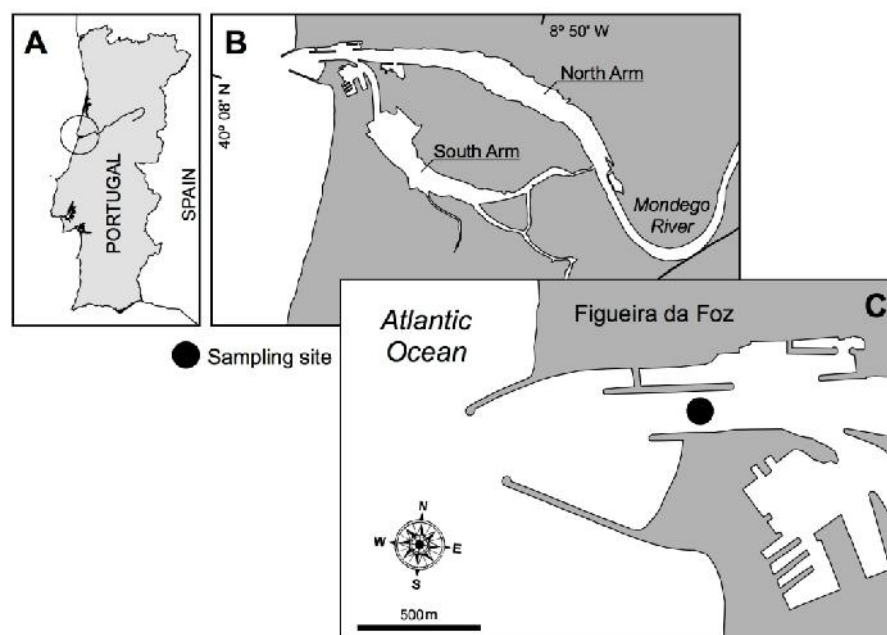


Figure 2. Location of the sampling site at the Mondego estuary.

At the laboratory, zooplankton samples were carefully cleansed with fresh water and transferred to 70% alcohol. Before being sorted, the contents of each bottle were rinsed in a sieve of the same mesh size as the net used in the field, to remove any debris.

2.2 Biomass determination

Organisms were examined in a Bogarov tray using a stereoscopic microscope, and separated by taxonomic group into small previously weighed aluminum capsules. Samples with very high numbers of individuals were sub-sampled as necessary by using a Folsom plankton splitter. Subsequently, the capsules were placed in heat resistant acrylic multiwell trays and put in a drying oven set to 60°C for at least 24 h, as recommended (Lovegrove 1962, 1966). This assured water removal without affecting organic content (Ré *et al.*, 2005). Finally, the capsules were weighed using an electronic micro-balance with an accuracy of 0.1 mg and values were annotated.

Zooplankton abundance values were obtained from dry mass calculated by subtracting the initial weight of empty aluminum capsules from the values registered after removal from the drying oven. These were converted into biomasses (mg m^{-3}) by division with the initial volumes and fractionation of samples. Conversion to carbon weight (mg C) was done assuming that it was 40% of dry weight, according to the methods employed by Omori & Ikeda (1984) and Båmstedt (1986). These values represent a realistic estimate of biomass and the nutritive value of zooplankton (Ré *et al.*, 2005).

2.3 Estimation of secondary production

Secondary production was calculated by following the temperature-dependent predictive method of Huntley & Lopez (1992). These authors have shown that for marine copepods, the temperature dependence of growth rates surpasses species differences. As a result, the exponential growth model presented should be applicable to production estimates of entire communities, regardless of species composition or

size frequency. In this study, growth was directly estimated from the weight of adult organisms for each taxonomic group and recorded temperatures. Next, the production rate was determined with the following equation:

$$P = B g,$$

where P is the production ($\text{mg C m}^{-3} \text{d}^{-1}$), B is biomass (mg m^{-3}), and g is the weight-specific growth rate ($\text{mass mass}^{-1} \text{d}^{-1}$).

2.4 Statistical analysis

Permutational univariate analysis of variance (PERMANOVA, Anderson 2001) was used to test for significant differences in both environmental and biological variables. Tests were applied to non-transformed data, and based on Euclidian distances between samples, considering all the factors as fixed and with unrestricted permutation of raw data.

Environmental variables were tested with a two-way design (season/depth) and seasonal variations in zooplankton biomass by a 1-way design (season). Copepod vertical distribution of biomass and production were tested for each season separately by two-way design (diel/depth and tide/depth). PERMANOVA tests were applied with PERMANOVA+ for PRIMER software (PRIMER v6 & PERMANOVA +v1, PRIMER-E Ltd.).

To identify which environmental variables influenced copepod production, Generalized Linear Models (R software; R Development Core Team, 2008) were applied. Again, each season was analyzed separately and environmental variables included were: salinity, temperature, chlorophyll a , and suspended particulate matter. Tide, diel cycle and depth were included as nominal variables, as well as interactions tide: depth, and diel: depth. Significant results in interaction terms indicate that the relationships between the explanatory variables are not the same for each condition. Variables were transformed whenever necessary (presence of extreme values): log transformation of Chl a and SPM in summer, winter and spring; square root transformation of secondary production in spring. The Spearman correlation coefficient was used to test for collinearity between continuous variables, and variables were excluded whenever the values

were superior to 0.7. A Gaussian error distribution was used for the response variables (copepod production). The selected variables included in the final model were obtained using an automatic stepwise selection (drop 1), and the Akaike information criterion (AIC, 1974) was used to select the best model.

Chapter 3

RESULTS

3.1 Environmental characterization

Salinity and water temperature revealed a marked seasonal variation typical of temperate estuaries (Fig. 3). Salinity ranged between 18.2–34.2 in summer, and 34.2–36 in autumn. Water temperature varied from 11.2–16.1°C in spring and 15.5–16.5°C in autumn. There were significant differences in the water temperature values between all seasons (Table 1, pseudo-F = 293.90, $p < 0.05$), while salinity displayed considerably higher values in spring and summer (pseudo-F = 31.86, $p < 0.05$). A similar pattern of variation was found for SPM, which presented significantly higher values in warmer seasons ($0.028 \pm 0.015 \text{ mg m}^{-3}$ spring, $0.05 \pm 0.03 \text{ mg m}^{-3}$ summer, pseudo-F = 25.01, $p < 0.05$). Chlorophyll *a* concentration increased noticeably in spring ($2.521 \pm 0.571 \text{ mg m}^{-3}$) and autumn ($2.9 \pm 0.945 \text{ mg m}^{-3}$) (Pseudo-F = 9.41, $p < 0.05$).

A vertical variation of environmental parameters in the water column was also detected (Fig. 4). Mean salinity values were generally higher at the bottom during summer and autumn (post hoc *t*-test, $p < 0.05$), showing no differences for the remaining seasons. At the surface, a sharp decrease in salinity was noted in autumn (31.4 ± 4.1) and winter (6.7 ± 3.8), indicating the occurrence of freshwater intrusion.

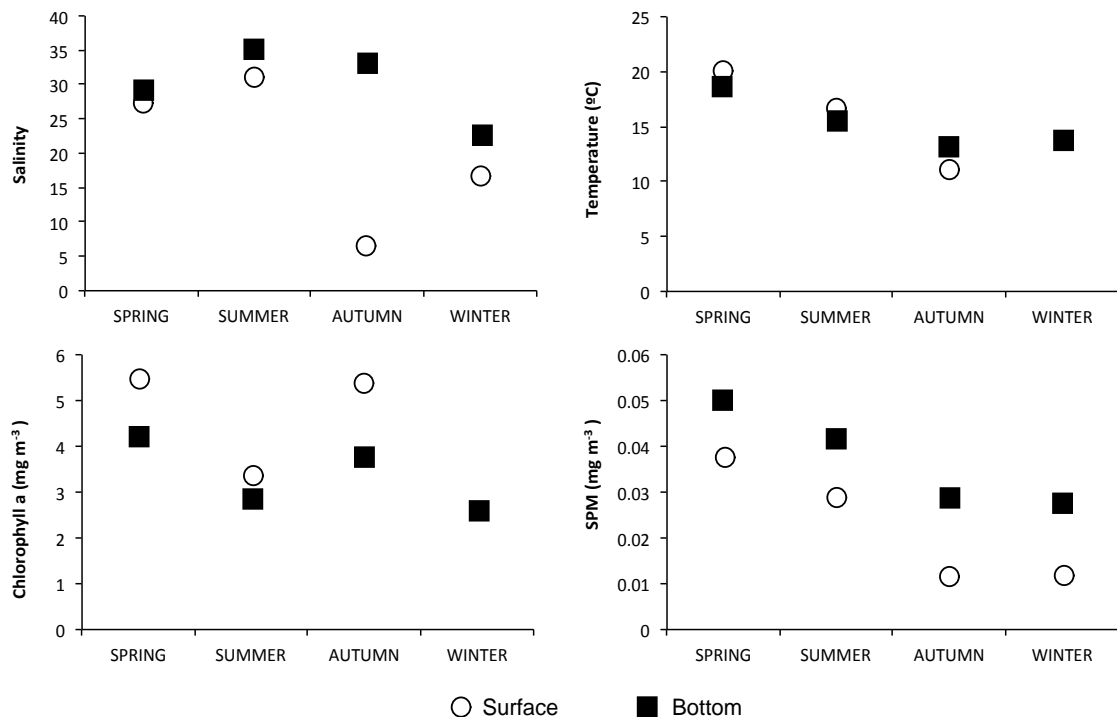


Figure 3. Vertical variation of environmental parameters recorded during the sampling period (mean values) in the Mondego estuary.

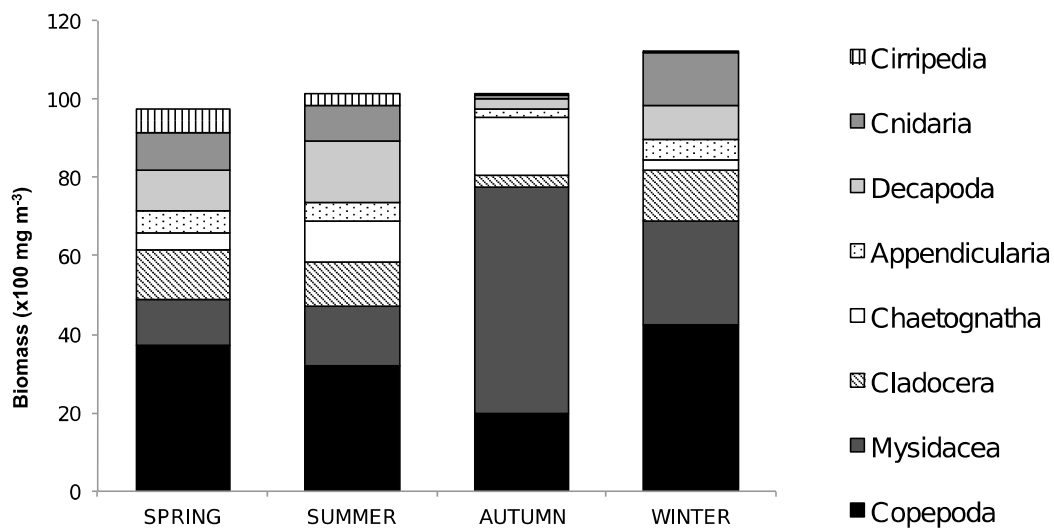


Figure 4. Mean zooplankton biomass ($\times 100 \text{ mg m}^{-3}$) by season for the main taxonomic groups present during the study period in the Mondego estuary.

Concerning temperature, autumn presented significantly higher values for the bottom, while values increased at the surface during spring (post hoc *t*-test, $p < 0.05$). Chlorophyll *a* concentration was always higher at the surface (pseudo-*F* = 4.85, $p < 0.05$) in opposition to the SPM load, which had higher values at the bottom (pseudo-*F* = 32.69, $p < 0.05$).

3.2 Seasonal, diel and tidal variations of biomass and secondary production

Gathered zooplankton organisms were identified in a total of 8 taxonomic groups (Fig. 5). Copepoda, Mysidacea, and Cladocera were the main taxa collected, contributing to 69% of the total biomass. Copepods dominated biomass during spring ($0.585 \pm 0.671 \text{ mg C m}^{-3}$), summer ($0.287 \pm 0.383 \text{ mg C m}^{-3}$), and winter ($0.221 \pm 0.128 \text{ mg C m}^{-3}$), while Mysidacea was the main contributor in autumn.

For total zooplankton biomass, no differences were observed between seasons. Nevertheless, Chaetognatha had significantly higher values in summer and autumn (pseudo-*F* = 3.55, $p < 0.05$), Mysidacea in autumn (pseudo-*F* = 2.58, $p < 0.05$), and Cirri-

pepida in summer and spring (pseudo-F = 9.31, $p < 0.05$) (Fig. 5). In general, copepod biomass was higher in winter, followed by spring, summer and autumn.

The diel and tidal vertical variations of copepod biomass are shown in Figures 6 and 7, respectively. Diel variation occurred mainly in spring (pseudo-F = 10.70, $p < 0.05$) and winter (pseudo-F = 8.31, $p < 0.05$), with substantially higher biomass values being registered at night for both the surface and bottom (Fig. 4). Moreover, Copepod biomass was consistently higher at the bottom during winter (pseudo-F = 6.27, $p < 0.05$) (Fig. 6).

Regarding tidal phases, no significant differences were observed between flood and ebb tides, even when considering vertical distribution, bottom and surface values (Fig. 7). In winter, bottom samples presented higher copepod biomass, both at ebb and flood tides (pseudo-F = 4.99, $p < 0.05$) (Fig. 7).

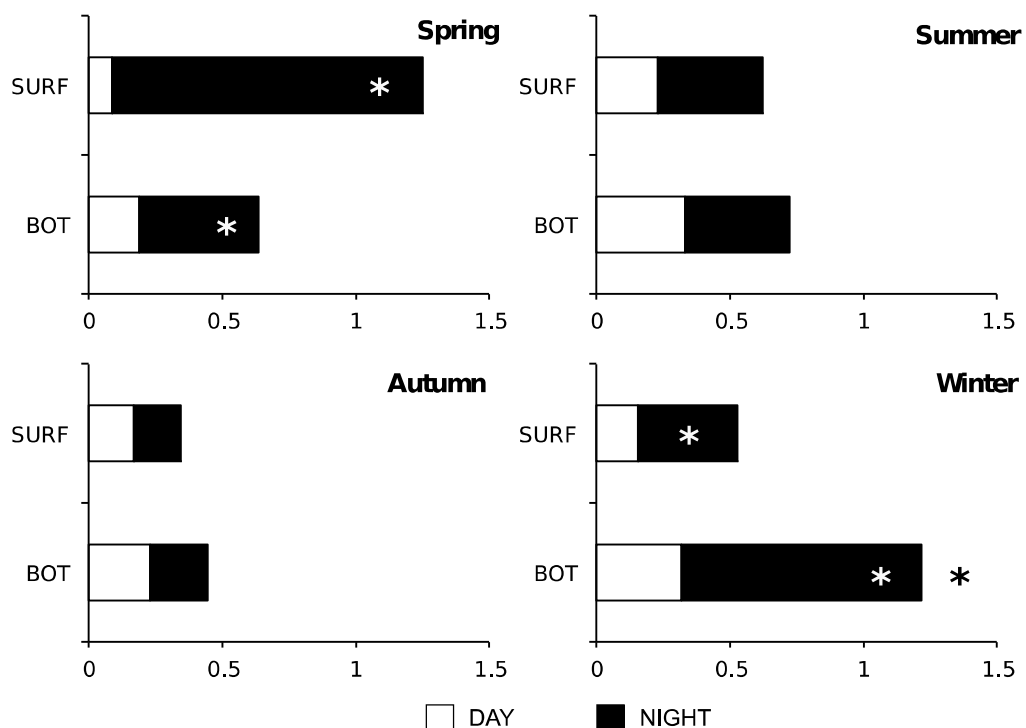


Figure 5. Diel vertical distribution of Copepoda biomass (mg m^{-3}) during the study period in the Mondego estuary. * Significant differences ($p < 0.05$).

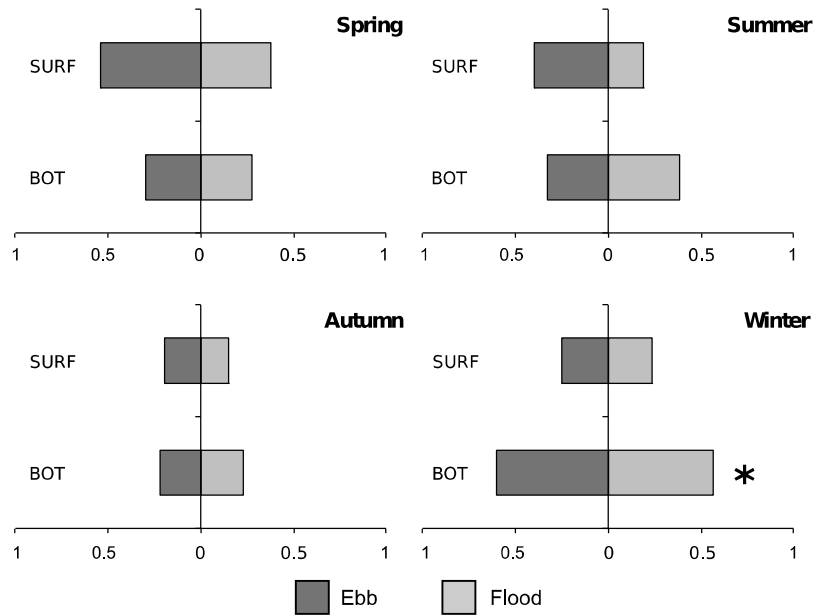


Figure 6. Tidal vertical distribution of Copepoda biomass (mg m^{-3}) during the study period in the Mondego estuary. * Significant differences ($p < 0.05$).

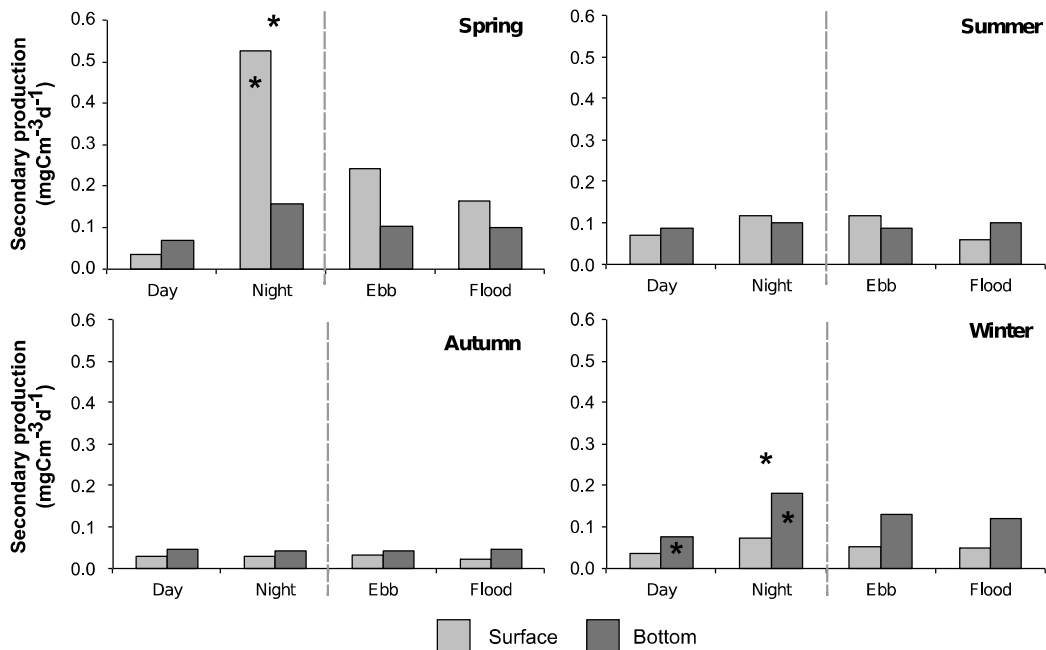


Figure 7. Diel and tidal vertical distribution of Copepoda production ($\text{mg C m}^{-3} \text{d}^{-1}$) during the study period in the Mondego estuary. * Significant differences ($p < 0.05$).

Secondary production values for copepods were much lower during autumn ($0.093 \pm 0.055 \text{ mg C m}^{-3} \text{d}^{-1}$, Pseudo-F = 2.67, $p < 0.05$) (Fig. 8). Daily secondary production rates exhibited significant differences in spring and winter, for both diel and depth

factors. Throughout winter, the highest values were found at night ($0.036 \pm 0.028 \text{ mg C m}^{-3} \text{ d}^{-1}$, Pseudo-F = 5.25, $p < 0.05$), and in the bottom samples ($0.044 \pm 0.027 \text{ mg C m}^{-3} \text{ d}^{-1}$, Pseudo-F = 2.96, $p < 0.05$). In spring, diel variation changed according to depth (Pseudo-F = 4.68, $p < 0.05$). The surface presented considerably higher values during the night (post hoc *t*-test, $p < 0.05$), while at the bottom there were no noteworthy differences. Copepod production showed no variation considering tidal phases.

3.3 Relationship between copepod production and environmental factors

From the GLM analyses run for each season, chlorophyll *a*, diel and depth were significant factors for the models (Table 1, Fig. 8). Spring and winter displayed higher coefficients of determination, explaining around 29% and 21% of the data variations (Table 1). Chl *a* had a linear negative effect on secondary production during summer, and a positive effect through autumn (Fig. 7).

Table 1. Season GLM results. Significant ($p \leq 0.05$) environmental variables marked (X); SPM, Suspended Particulate Matter. (-) Excluded variables due to collinearity.

	Spring	Summer	Autumn	Winter
Salinity				
Temperature		(-)	(-)	
Chlorophyll <i>a</i>		x	x	
SPM			(-)	
Diel	x			x
Tide				
Depth	x			x
Diel:Depth	x			
Tide:Depth				
F-statistic	5.786	3.287	8.319	5.418
p-value	0.002	0.080	0.006	0.008
R²	0.29	0.09	0.18	0.21

Along spring and winter, secondary production varied according to diel phase and depth. In winter, night and bottom samples revealed consistently higher daily secondary production rates. However, during spring this relation changed, showing a significant interaction between factors: daytime secondary production is higher at the bottom, while at night production is higher at the surface (Fig. 8).

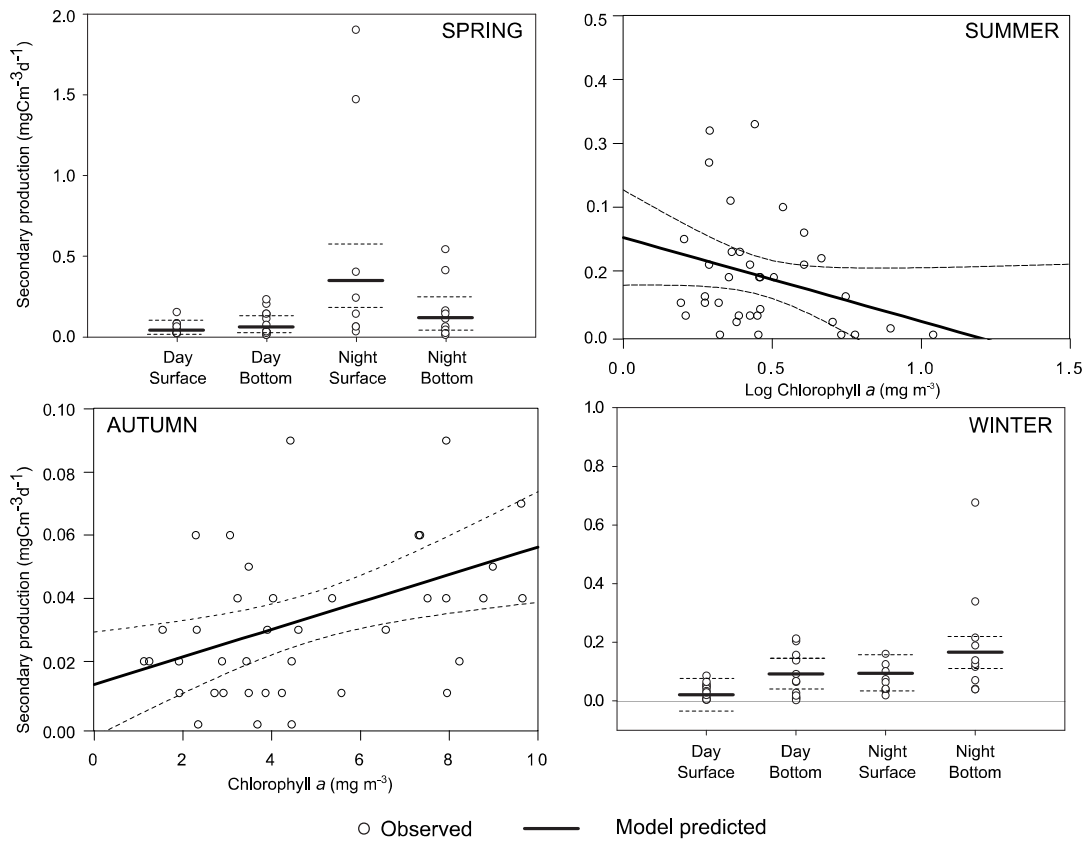


Figure 8. Generalized linear models fitted to secondary production at each season. Only significant variables at $p < 0.05$ are shown. Dashed lines are approximate 95% confidence intervals.

Chapter 4

DISCUSSION

4.1 Community composition

Zooplankton is composed of animals from several taxonomic groups, but crustaceans are generally dominant. In terms of the overall contribution to zooplankton biomass, the most important group found during this study was Copepoda. This is in accordance with previous studies of zooplankton community abundance and diversity in the Mondego estuary (Marques *et al.*, 2006). In fact, total zooplankton abundance reflected the seasonal variation of the copepod population quite well. The results are also parallel to findings in other areas, which revealed that copepods usually contribute with the majority of zooplankton biomass and species diversity in estuaries (Uye & Liang 1998, Arashkevich *et al.* 2002, Leandro *et al.*, 2007). Other organisms encountered included gelatinous species (consisting primarily of medusae, chaetognaths, and appendicularians). Due to low dry weight, their contribution to biomass was minimal. Nevertheless, their total predatory impact on the zooplankton community is possibly important, since these carnivores have high grazing rates (Purcell *et al.* 1994; Marshalonis & Pinckney, 2008). When numerous, they significantly affect plankton abundance and size composition.

4.2 Biomass and secondary production

Although estuarine ecosystems are generally characterized by high zooplanktonic biomass and secondary production values (David *et al.*, 2006), the results obtained for the Mondego estuary are lower than those encountered in the literature for other regions, such as the Ria de Aveiro Portugal (Leandro *et al.*, 2007), Westerschelde Netherlands (Escaravage & Soetaert, 1995), Kattegat Denmark (Kjørboe & Nielsen, 1994), and the Inland Sea of Japan (Uye & Liang, 1998). These authors found numbers ranging from a minimum of $1.18 \text{ mg C m}^{-3} \text{ d}^{-1}$ (Kattegat) to a maximum of $6.85 \text{ mg C m}^{-3} \text{ d}^{-1}$ (Inland Sea of Japan). Mean production for the Mondego estuary was only $0.125 \text{ mg C m}^{-3} \text{ d}^{-1}$ when considering the contribution of copepods alone (main taxonomic group).

Methods for zooplankton biomass estimation are relatively standard, and calculated values have been reported for different areas worldwide (Kimmerer & McKinnon,

1987; Roman *et al.*, 2002; Fernández de Puellas *et al.*, 2003; Rawlinson *et al.*, 2004; Irigoien & Castel, 2005; Hays *et al.*, 2001; Melo Júnior *et al.*, 2007). However, data are generally obtained from samples collected with nets of mesh diameter ranging from 64 to 300 μm . It is important to note that net selectivity could have a significant influence on generated data, since it can differentially represent the dominant species and/or the proportion of developmental stages present in the environment. Most coastal mesozooplankton assemblages are composed of small-sized organisms, as well as early developmental stages of larger species, which generally dominate in terms of abundance and biomass (Turner, 2004). Consequently, the differences observed in this study were probably due to the higher mesh size used in comparison to other investigations (64 and 125 μm – Ria de Aveiro, 55 μm – Westerschelde, 50 μm – Kattegat and 62 μm – Inland Sea of Japan). More importantly, the frequent undersampling of small copepod species may lead to a limited view of the planktonic systems and must be considered when comparing results. In this respect, the importance of small copepods is well recognized as a fundamental link in marine food webs, serving as major grazers of phytoplankton and prey for ichthyoplankton and other larger pelagic carnivores (Verity *et al.* 2002; Turner, 2004). While it is now recognized that many factors contribute to fish recruitment (Houde, 2008), the amount of zooplankton prey available to larval fish remains without a doubt vital.

Concerning the estimation of secondary production rate, the selected methodology may also represent a problem in the process of assessment. The current lack of a completely accepted method for determining secondary production is a consequence of the variety of factors affecting secondary production rate, with difficulty in precisely determining the efficiency of each one. Moreover, in pelagic research efforts on zooplankton growth rates are mainly focused on copepods (Hirst & McKinnon, 2001). As mentioned earlier, several methods can be used to determine secondary production for zooplankton, but in addition to biomass values, all of them require the determination of growth rates (Leandro *et al.*, 2007) in addition to other variables. With this background in mind, and considering the nature of the preserved samples, available field parameters, and the lack of more detailed information, it was decided that the mathematical growth model described by Huntley & Lopez (1992) would be the most

appropriate to conduct the intended analysis. This model depends only on temperature, and does not assume that copepod/zooplankton growth may be food limited at some points during the year (Burkill & Kendall, 1982; Peterson *et al.*, 1991). Nevertheless, caution must be used in its application, as it tends to overestimate growth rates (Kleppel & McKinnon, 1996). Lastly, it is important to understand that this type of model is a reliable tool for estimating secondary production in the Mondego estuary, since it considers ecologically relevant parameters such as biomass and growth rate, which have been more extensively studied.

4.3 Relationship between copepod production and environmental factors

During the study period, both copepod biomass and production rate displayed a weak seasonal effect. This lack of cyclic variation may be related to species replacement when they are seasonally substituted by each other, yet maintain average biomass (e.g. freshwater species replaced by marine ones). Changes in copepod secondary production were only detected in autumn, which had the lowest values. During autumn, the increased prevalence of predator species (e.g. Mysidacea and Chaetognatha) most likely influences copepod community structure by favouring species with lower growth rates. Salinity values detected at the surface during this season indicated a strong input of freshwater that may also affect the planktonic community. According to Kimmerer (2002), variations on estuarine organisms' abundance and survival may occur through attributes of physical habitat that vary with flow, and negative responses can be expected mainly from marine species due to the seaward displacement of their habitat, and therefore, population centers.

During autumn, the low production rates were positively related with chlorophyll *a*, showing no diel, tidal, or vertical patterns. The same occurred during summer, although presenting an inverse (negative) relationship with primary productivity. These results further suggest a seasonal shift in the copepods' diet in the estuary. Contrasting with autumn, which exhibited high values of chlorophyll *a*, the amount of primary production in summer in the Mondego estuary was quite certainly well below the carbon

requirements of the copepod population, not being sufficient enough to cover their nutritional needs. Phytoplankton appears to be the most important carbon source over the course of autumn, while in summer alternative sources are utilized. Many researchers have noted the paradox between high copepod biomass in estuarine areas with a high abundance of particles and low phytoplankton production (David *et al.*, 2006). This suggests the possible use of detritus (essentially originating from terrestrial plants) as a food source for estuarine copepods. For instance, Diodato & Hoffmeyer (2008) found that *A. tonsa*, the most abundant species in the Mondego estuary, consumed detritus in the Bahía Blanca Estuary (Argentina), and a study of the cycling of organic material in the Kariega Estuary (South Africa) revealed that mesozooplankton used both phytoplankton and detritus as food (Richoux & Froneman, 2008).

4.4 Diel, vertical and tidal patterns

In spring and winter, GLM analysis identified diel and vertical patterns as having a key role on production rate variation, as opposed to summer and autumn. In fact, during spring and autumn, both biomass and production rates were significantly higher at nighttime than at daylight hours. Additionally, spring copepod production rates seem to show a vertical variation associated with depth (changes in depth according to diel phase); a strong evidence of diel vertical migrations. It is generally accepted that the daily sunlight cycle plays an important role in the DVM behaviour of zooplankton, which has been observed in coastal regions (e.g. Zhu *et al.* 2000; Devreker *et al.* 2008, Marques *et al.* 2009; Primo *et al.* 2012). Nocturnal vertical movements in the water column have commonly been reported for different taxa, including copepods (Rawlinson *et al.* 2005). Synergistic benefits might have shaped the evolution of this adaptive complex behaviour in response to variations in the environment. Explanations for the vertical migration mechanism include environmental factors (e.g. light, temperature, salinity, oxygen, and diet), predator-avoidance, and energy and resource utilization (for a detailed review sees Hays, 2003). This behaviour causes active transport of dissolved organic and inorganic carbon and nitrogen, and plays a well-documented role in biogeochemical cycling (Steinberg *et al.* 2002). Furthermore, there are associated consequences for higher trophic levels of this behaviour. For example,

Hays (2003) noted that some predators at higher trophic levels modify their activity to exploit the vertical movement of the food source. The food chain of the Mondego estuary 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 community, concluded that copepods were an important component of the fish diet. As a result, DVM behaviour could lead to important trophodynamic effects in the fish community.

Vertical patterns were stronger during winter, where bottom samples showed increased biomass and production rates compared to surface samples. Despite autumn samples presenting higher salinity stratification, during winter salinity at the surface in the Mondego estuary is highly variable due to changes in freshwater flow (Marques *et al.*, 2009). The instability of the water column influences planktonic organisms to gather near the bottom in order to avoid being flushed out of the estuary.

Contrary to diel and vertical distributions, variations in biomass and secondary production showed no relation with tides. This can indicate that the amount of organisms imported (flood) was equivalent to those exported (ebb) from the estuary. However, results from vertical salinity variation pointed out low stratification during spring and summer. At this time a persistent landward current seemed to occur, increasing the import of biomass and transportation to upstream areas. The higher freshwater flow detected in autumn and its strong associated advective effects (export) are mainly seen at the surface, but the same amount of biomass and production rate appear to be inputted near the bottom, hence the absence of vertical differences. During winter, copepods tend to agglomerate close to the bottom, which results in a lower transport of organisms.

Chapter 5

CONCLUSION

5.1 General conclusions

This study represents the first attempt at estimating biomass and secondary production of the zooplankton community of the Mondego estuary, focusing on Copepoda which represent 60% of the total biomass. The considerable complexity in the processes structuring the dynamics and composition of biomass and production rates has been shown. The Mondego did not display the typical seasonal patterns of high zooplankton biomass in summer and lows in winter common to similar sites (Fernández de Puelles *et al.*, 2003; Rawlinson *et al.*, 2004). Nonetheless, in the western Mediterranean basin the range of zooplankton biomass and abundance are not always well defined, and comparisons between data of other systems should be done carefully because of the great variety of sampling methods that have been used. Tidal exchange also revealed a non-significant variation, with little difference between imported and exported biomass and production. This is not uncommon, and studies in other locations have yielded equivocal results with different explanations, as discussed by Melo Júnior (2007). In a shallow coastal ecosystem like the Mondego estuary, the influence of river discharge and varying salinity levels are significant, and it is possible to conclude that local factors (freshwater flow, predation, food limitation) regulate the seasonal variations in zooplankton biomass and production.

In the future, it would be important to conduct this type of work using nets of a smaller mesh size in order to estimate the contribution of smaller sized zooplankton (e.g. nauplii, copepodites, developmental stages of crustaceans), which have been undersampled and underestimated in this study. Naupliar stages and smaller copepods have been shown to dominate zooplankton populations in terms of abundance, biomass, and productivity, and are therefore of crucial importance. It would also be interesting to define a copepod growth model specific to this ecosystem in order to achieve more accurate production estimates.

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