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**COPEPOD SECONDARY PRODUCTION
RESPONSE TO CLIMATE VARIABILITY IN
THE MONDEGO ESTUARY**

**Dissertation under the Masters of Ecology supervised by Professor
Doctor Miguel Ângelo do Carmo Pardal and by Doctor Ana Lúcia
Sequeira Primo presented to Department of Life Sciences of the Faculty
of Sciences and Technology of the University of Coimbra**

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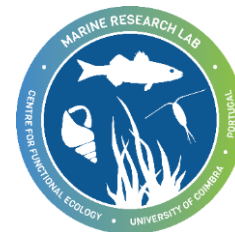
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“What we most want to find in our lives will be found in the places where we least want to look”

- **Carl Jung**

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ABSTRACT

This work aimed to evaluate the response of copepod secondary production to climate variability in the Mondego estuary (Portugal, Iberian Peninsula), over the period of 2003 to 2012. To achieve this goal, monthly samples were collected along the estuarine gradient. Zooplankton biomass and secondary production spatiotemporal variability were analysed with an emphasis on the copepod community. Seasonal, interannual and spatial distributions were evaluated and the influence of physical processes operating at different temporal scales analysed.

Main results showed that species distribution along the estuary was related to river freshwater input and/or with the marine intrusion of seawater, also marine and estuarine copepods' biomass and production exhibited an absence of seasonality during the studied period. In general, copepod biomass and production interannual variability displayed a strong relation with extreme events, which nowadays are increasingly common. It was possible to identify a significant increasing long-term trend in biomass and production for marine and estuarine copepods. Furthermore, these copepods' biomass and production displayed positive correlations with planktonic predators, while for freshwater copepods they showed negative correlations with salinity, river flow and temperature. Higher prevalence of marine copepods, in response to greater salinity in the Mondego estuary, may lead to shifts in the community structure inducing changes at upper trophic levels, including commercially important nursery species.

Keywords: Long-term trends; Extreme events; Productivity; Zooplankton; Estuarine systems; Climate change.

Este trabalho teve como objetivo avaliar o impacto da variabilidade climática na produção secundária de copépodes no estuário do Mondego (Portugal, Península Ibérica), no período entre 2003 e 2012. Para isso amostragens mensais foram efetuadas ao longo do gradiente estuarino em cinco/seis estações de amostragem. A variabilidade temporal e espacial da biomassa e produção secundária zooplanctónica foi analisada, com um maior foco na comunidade de copépodes. Distribuições sazonais, inter-anuais e espaciais foram investigadas, simultaneamente a influência dos processos físicos em várias escalas foi também analisada.

Os principais resultados mostram que durante a distribuição das espécies ao longo do estuário está relacionada com o input de água doce através do rio e/ou intrusão de água marinha, e que os copépodes de espécies marinhas e estuarinas não apresentaram sazonalidade durante o período estudado. No geral, a variação inter-anual da biomassa e produção de copépodes apresentam uma forte ligação com a existência de eventos extremos, que hoje em dia são cada vez mais comuns. Foi possível identificar uma tendência significativa de aumento a longo prazo da biomassa e produção para as espécies de copépodes marinhos e estuarinos. A biomassa e produção dos mesmos mostraram uma correlação positiva com predadores planctónicos, enquanto no caso dos copépodes de água doce, a biomassa e produção mostraram correlações negativas com a salinidade, o fluxo de água do rio e a temperatura. Uma maior prevalência de copépodes marinhos, em resposta ao aumento da salinidade no estuário do Mondego, pode levar a mudanças na estrutura da comunidade afetando níveis tróficos superiores, incluindo espécies de peixe comercialmente importantes.

Palavras-Chave: Tendências a longo prazo; Eventos extremos; Produtividade; Zooplâncton; Sistemas estuarinos; Alterações climáticas.

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CHAPTER 1 – INTRODUCTION

1.1. Long-term data sets as tools to assess changes in ecological communities

Long-term data sets are repeated field-based empirical measurements that are continuously collected as a result of monitoring efforts of at least 10 years (Lindenmayer & Likens, 2010). They are important to evaluate changes in ecosystem structure and function while also providing critical insights into changes in ecosystem services (Lindenmayer & Likens, 2010).

Ecological monitoring forms the basis of many long-term scientific strategies for marine ecosystems management (Edwards et al., 2010). These programmes have experienced a renaissance since the 1990s, and have been fundamental to document, understand and predict the impacts of climate change on ecosystems (Doney et al., 2012; Ducklow et al., 2009; Lindenmayer & Likens, 2010; Richardson, 2008). Multi-decadal periods are often needed to distinguish climate change signals from substantial natural variability (e.g., regime shifts) (Doney et al., 2012; Ducklow et al., 2009). When a monitoring programme expands in time and spatial scale, it captures more spatiotemporal structures and natural phenomena, as well as decadal and multi-decadal shifts in baselines and regimes, providing an ideal way to separate anthropogenic and natural biological signals and to address the extent and severity of these impacts (Edwards et al., 2010).

1.2. Zooplankton – definition, importance, and environmental forcing

Zooplankton correspond to the heterotrophic part of plankton and include animals from several taxonomic groups (Hays et al., 2005; Hirst, 2017), with copepods being generally dominant (Feinberg & Dam, 1998), comprising up to 80% of the mesozooplankton biomass (Verity & Smetacek, 1996).

They are the link between producers and higher trophic levels representing a key ecological role in capturing and transferring energy while modulating the carbon-flow processes through their interactions with both levels (Gonçalves et al., 2015; Primo et al., 2009; Richardson, 2008). In addition, zooplankton are important indicators of change in aquatic systems due to: (1) being poikilothermic which makes their physiological processes highly sensitive to temperature (Mauchline, 1998; Richardson, 2008); (2) being short-lived, thus tight coupling of climate and population dynamics is possible; (3)

not being commercially exploited, hence not affected by exploitation (Hays et al., 2005; Richardson, 2008); (4) being able to show dramatic oscillations in distribution due to their rapid response to changes in temperature and oceanic currents, by expanding and contracting their ranges (Hays et al., 2005). Furthermore, evidence suggests that zooplankton are more sensitive indicators of change than environmental variables themselves (Hays et al., 2005; Taylor et al., 2002).

Zooplankton are influenced by factors acting on multiple dimensions, ranging from small- to large-scale (Beaugrand & Reid, 2003; Gonçalves et al., 2015). At a global scale, zooplankton dynamics (e.g., biomass, species composition, diversity, size structure and phenology) are notably correlated with large-scale modes of climate variability such as the North Atlantic Oscillation (NAO) (Beaugrand & Reid, 2003; Drinkwater et al., 2003; Fromentin & Planque, 1996; Greene et al., 2003), the El Niño Southern Oscillation (ENSO) (Chiba & Saino, 2003; McGowan et al., 2003) and/or the Pacific Decadal Oscillation (PDO) (Mantua et al., 1997). Further, on a regional scale, physical processes (e.g., currents, frontal structures, buoyancy-driven plumes, and coastal upwelling) govern the dispersion of individuals and the availability of nutrients (Garçon et al., 2001; González-Gil et al., 2015). At smaller scales, life-history traits of zooplankton (e.g., growth, fecundity, and survival) are mostly affected by environmental factors such as temperature (Huntley & Lopez, 1992), salinity (Cervetto et al., 1999; Gaudy et al., 2000; Jassby et al., 1995), food availability/quality (Hirst & Kiørboe, 2002; Paffenhöfer & Stearns, 1988), and biotic interactions (e.g., predation and competition) (Giske et al., 1990; Kimmel & Roman, 2004; Soetaert & Van Rijswijk, 1993).

Nowadays, various authors encourage the use of zooplankton long-term monitoring studies to provide valuable information about climate-ecosystem interactions (Chiba et al., 2018; Hays et al., 2005; Mackas et al., 2001; Mackas & Beaugrand, 2010). Zooplankton monitoring projects have helped to increase our understanding of the processes by which marine ecosystems respond to climate change and other environmental variations, additionally its scientific value is recognized in the contexts of fisheries, biodiversity, and global change studies (Chiba et al., 2018).

1.3. Climate change impacts on planktonic communities

Climate change refers to changes in the climate state that can be identified by changing mean and/or the variability of its properties, which persist for an extended period, typically decades or longer (Field et al., 2014). It may be due to natural internal processes or external forcings such as variations of the solar cycles, volcanic eruptions,

and persistent anthropogenic changes in atmosphere composition or land use (Field et al., 2014).

Climate change due to external human forcing has profound and diverse consequences for marine ecosystems. 93% of the extra energy arising from anthropogenic greenhouse gas emissions have been absorbed by the global ocean, resulting in an increase in average global surface temperatures since the beginning of the 20th century, approaching 1°C (0.89°C, over the period 1901-2012) (Stocker et al., 2013). These climbing temperatures create a multitude of secondary changes, such as rising sea level, increased ocean stratification, decreased sea-ice extent, and altered ocean circulation, precipitation, and freshwater input (Doney et al., 2012). In addition, the combining effect of warming and altered ocean circulation reduces subsurface oxygen (O₂) concentrations (Keeling et al., 2010). Furthermore, climate change is also expected to increase the frequency and intensity of climate extremes worldwide (Dolbeth et al., 2011; Gleick, 2003; Jentsch et al., 2007; Mirza, 2003; Solomon et al., 2007).

Coastal marine ecosystems' structure and functioning are harshly affected by global climate change (Harley et al., 2006; Houghton, 2005). Climate changes lead to physical and chemical modifications in the environment (e.g., temperature, salinity, freshwater flow), which alter biological processes, such as species physiology, phenology, and distribution (Hughes, 2000). Over recent decades considerable evidence for regular changes in plankton abundance and community structure linked to recent warming have arisen in many areas of the world (Hays et al., 2005; Poloczanska et al., 2016). Declines in cold-water species and increases in warm-water ones have been observed for mysids in Mediterranean caves (Chevaldonne & Lejeusne, 2003) and zooplankton communities in the north-east Atlantic (Beaugrand, 2003). Also, plankton range extension in response to climate warming has been documented in the north-east Atlantic, with some of the highest rates being observed for zooplankton (Poloczanska et al., 2016). In addition, it seems that the planktonic community in the central North Sea is responding to climate change by advancing/delaying seasonal maximum peaks, a response differing according to the species and functional groups. These diverse responses to climate change throughout the community may lead to mismatches between trophic levels and functional groups, possibly leading to severe implications on commercial fisheries (Hays et al., 2005).

Developing a greater capability for monitoring and understanding these changes will be critical for future management of ocean and coastal resources (Poloczanska et al., 2016). Increasing evidence suggests that we can use studies on plankton to predict the impact of these changes since these organisms play a pivotal role in trophic

dynamics and food webs stability (Hays et al., 2005; Taylor et al., 2002). Substantial changes in their structure or composition may engage bottom-up forcing with impacts on nutrient trophic cycling and fish recruitment (Beaugrand & Reid, 2003; Hays et al., 2005).

1.4. Estuarine areas

Estuaries are partially enclosed bodies of water where freshwater flows into the oceans, mixing with seawater, forming transition zones or ecotones from land to sea with a concurrent gradient from fresh to saltwater, and creating some of the most biologically productive areas on Earth (Kennish, 2002; McLusky & Elliott, 2004). These transition areas are characterized by intensive chemical, physical and biological processes leading to a considerable variety of inter-related biotic and abiotic structural components (Kennish, 2002; Paerl, 2006; Telesh, 2004).

These systems are recognized worldwide for their ecological and socioeconomic value, being among the most productive and valuable natural ecosystems (Costanza et al., 1998; Dolbeth et al., 2003; Kennish, 2002; Paerl, 2006). Often referred to as nurseries, estuaries play a crucial role in many species' lifecycles, providing shelter and feeding grounds (Able, 2005; Beck et al., 2001; Elliott & McLusky, 2002).

Estuaries are relatively shallow coastal environments that are sensitive to environmental parameters, and are strongly affected by natural variations in climate, integrating the effects of the sea and the surrounding watershed (Marques et al., 2014, 2007; Primo et al., 2015). The specific vulnerability of an estuary depends on its physical properties, geomorphology, and biota (Attrill & Power, 2000; Marques et al., 2007; Scavia et al., 2002).

Worldwide, estuarine ecosystems have been affected by climate change, mainly through alterations in freshwater input, increasing air and water temperature, shifts in connectivity to the sea and increased intrusion of marine waters (Chaalali et al., 2013; Goberville et al., 2010). At the Gironde estuary, salinity and temperature were related to the upstream progression of several neritic species (Chaalali et al., 2013). Similarly, in the Mondego estuary, wide hydroclimate changes were experienced in past years, including shifts in precipitation regimes (Marques et al., 2014), that fostered drought conditions and produced prolonged periods of low freshwater inflow (Marques et al., 2018), resulting in higher saline intrusion (Leitão et al., 2007; Martinho et al., 2007). The effects induced by droughts (higher saline intrusion), in the Mondego estuary, reportedly affected several components of the community as fish (Dolbeth et al., 2008; Martinho et al., 2007; Nyitrai et al., 2012), zooplankton (Marques et al., 2014, 2007; Primo et al.,

2009, 2015) and ichthyoplankton (Primo et al., 2011), causing an overall gradual replacement of freshwater communities, in the upper reaches of the estuary, by more salinity tolerant ones.

Concerning the warming of surface waters, in several European estuarine systems, a disappearance of cold-water fish species in favour of species with sub-tropical and tropical affinities has been reported (Cabral et al., 2001; Nicolas et al., 2011; Vinagre et al., 2009). Succinctly, the immediate effects of climate forcing in estuaries, foster modifications in their structure and function, also their direct exposure to anthropogenic influences (Elliott & Kennish, 2011; Kennish, 2002), warns of synergistic interactions of climate and anthropogenic forces that could magnify these effects (Hidalgo et al., 2011; Wiedenmann et al., 2013).

1.5. Secondary production

Secondary production has been historically defined as the production of herbivorous organisms, therefore being the second trophic level after primary producers (Clarke, 1946). However, recent studies indicate that it is best to think of it as 'secondary' in the sense that it is heterotrophic, and ultimately dependent upon primary production (Hirst, 2017). This definition allows for the implied fact that biomass may either accumulate or be transitory (passing to trophic levels or decaying). Hence, secondary production is the amount of organic matter produced per unit of time (e.g., days and years) per a unit of space (e.g., area and volume), regardless of its fate (Clarke, 1946; Cusson & Bourget, 2005; Kimmerer, 1987).

In aquatic systems, the production of biological components is of interest as a measure of energy flow, through a population and as an indicator of its physiological or nutritional state (Dolbeth et al., 2012; Gonçalves et al., 2015; Kimmerer, 1987).

Secondary production is valuable in decision making, allowing for rational management of biological resources. Acquired estimates often represent a direct measure of food provision delivered by an ecosystem, being relevant socio-economically, especially when attached to a monetary value (Dolbeth et al., 2012). This condition is desired when assessing ecological processes and resources, as it is attractive and well understood by policymakers and the general public (Costanza et al., 1998; Pinto et al., 2010). Furthermore, secondary production is important for the assessment of environmental impacts, providing insight into ecosystem dynamics, as it combines both static (e.g., biomass and density) and dynamic components (e.g., growth

and reproduction) of a population's ecological performance, and therefore it aids in detecting ecological changes (Dolbeth et al., 2012).

Zooplankton secondary production straightforwardly is the sum of all growth (somatic and reproductive) (Yebra et al., 2017) combined with biomass losses (e.g., mortality rates and emigrations) over a given period (Downing & Rigler, 1984; Yebra et al., 2017). Kimmerer (1987) revealed that the instantaneous zooplankton production rate could be determined as the product of total biomass and the weight-specific growth rate of a population. Subsequently, all estimating methods of zooplankton production involve the measurement of both biomass and weight-specific growth rate (hereafter referred to as growth rate) (Huntley & Lopez, 1992). Direct estimation of biomass is usually more straightforward than the growth rate since it can be directly estimated after sampling (e.g., dry weight), whereas growth requires repeated sampling and/or incubations (Lopes, 2007; Yebra et al., 2017).

Traditionally, growth and production rate measurements have focused on copepods, the main component of marine zooplankton, these 'copepod-centric' methods are based on either direct or indirect procedures (Yebra et al., 2017). Direct estimates of growth assess changes in weight or reproductive output (Downing & Rigler, 1984; Runge & Roff, 2000; Yebra et al., 2017). One of the first direct methods used for estimating *in situ* growth rates was the natural cohort technique (Heinle, 1966), however, this approach had problems when applied to marine zooplankton (Yebra et al., 2017). These issues were surmounted by using incubation techniques, such as egg production, moulting rate and artificial cohort methods, as they can be applied to populations that continuously reproduce (Yebra et al., 2017).

Indirect methods for measuring growth and production rates consist of modelling and biochemical approaches. The use of generalized models of growth has the advantage that growth can be predicted from easily obtained *in situ* data, and no incubations are necessary (Yebra et al., 2017). Empirical models estimate growth from relationships coupled with environmental variables such as temperature and Chlorophyll *a*, and some characteristics of the zooplankton group studied such as body size, stage of development, spawning type, sex, and broad taxonomic grouping (Hirst & Bunker, 2003; Hirst & Lampitt, 1998; Huntley & Lopez, 1992; Yebra et al., 2017).

However, models are best applied to the species/groups used in their assembly and cannot be generalized, hence, empirical models may induce uncertainties when estimating growth rate (Hirst & Lampitt, 1998; Kobari et al., 2003; Lin et al., 2013; Runge & Roff, 2000). As alternatives to these traditional methodologies, other techniques using

biochemical measurements have been developed for the estimation of weight-specific growth rates, such as using radio-labelled compounds, nucleic acids, and enzyme activities as proxies for growth (Yebra et al., 2017).

In previous studies, Gonçalves et al. (2015) reported that the maximum secondary production value for the Mondego estuary was $0.125 \text{ mg C m}^{-3} \text{ d}^{-1}$ when only considering copepods (main taxonomic group). According to these authors, this value was lower than the ones stated for other regions, such as the Ria de Aveiro in Portugal (Leandro et al., 2007), the Inland Sea of Japan (Uye & Liang, 1998), Westerschelde in the Netherlands (Escaravage & Soetaert, 1995), and Kattegat in Denmark (Kjørboe & Nielsen, 1994), with values ranging from $1.18 \text{ mg C m}^{-3} \text{ d}^{-1}$ (Kattegat) to $6.85 \text{ mg C m}^{-3} \text{ d}^{-1}$ (Inland Sea of Japan). These authors justified the differences observed, by the usage of a larger mesh size ($335 \mu\text{m}$) in comparison with other studies ($50 - 125 \mu\text{m}$).

Secondary production in the Mondego estuary seems to be affected by extreme weather events, such as droughts and floods, affecting the production of the key species such as *Hydrobia ulvae* (Cardoso et al., 2008), marine migrant and resident fish species (Bento et al., 2016; Nyitrai et al., 2013). According to Dolbeth et al. (2008, 2011), these extreme weather events had implications for the food provisioning of the estuary, since the production of commercially important species (shellfish and fish) declined after them.

In other regions, Sheridan & Landry (2004), in the North Pacific subtropical gyre, and Steinberg et al. (2012), in the North Atlantic subtropical gyre, described an increase in mesozooplankton biomass, most likely promoted by bottom-up control induced by increasing pico-phytoplankton, translating up the microbial food web into mesozooplankton. Nonetheless, this scenario is not predicted for increasingly stratified subtropical oceans in which nutrient exchange is limited and production declines (Behrenfeld et al., 2006; Doney, 2006). Therefore, it is possible that for a period climate warming has increased primary and secondary production, and at some future date, this trend will reverse (Steinberg et al., 2012).

In the case of Piontkovski & Castellani (2009), a nearly 10-fold decrease in mesozooplankton biomass occurred in the tropical North Atlantic, which has been attributed to decreases in primary production due to a thinning of the mixed layer as a result of surface-ocean warming. This agrees with the findings of Wiafe et al. (2008), reporting a 4-fold decrease in zooplankton biomass in the eastern tropical Atlantic.

1.6. Objectives

Global ecological changes are occurring at a very large frequency and scale, therefore, understanding how these will influence the planet is a key issue worldwide (Hays et al., 2005; Poloczanska et al., 2016; Walther et al., 2002). Unravelling climate change effects require integrating impacts from several stressors, highlighting the importance of implementing appropriate long-term monitoring schemes and adequate data mining (Dolbeth et al., 2012; Marques et al., 2018; Primo et al., 2015). This will help to anticipate ecological responses and will give insight into the long-term management of coastal resources (Cloern et al., 2016). Combining secondary production with long-term data sets upgrades the understanding of the integrity of ecosystem functioning (Dolbeth et al., 2007, 2011; Pranovi et al., 2008), for which the use of density or biomass fails to provide a broader overview of the potential impacts (Dolbeth et al., 2012). Therefore, long-term studies of secondary production can represent a fundamental tool to understand changes in aquatic ecosystems. In this way, the main goal of this study is to determine the impact of climate variability on the copepod secondary production of the Mondego estuary, while providing:

- a) Long-term estimates of copepod community production in an estuarine habitat.
- b) New understanding about the factors causing variability in copepod production.

CHAPTER 2 – MATERIALS AND METHODS

2.1. Study Site

The studied ecosystem was the Mondego estuary, it is situated on the western coast of Portugal (40°08' N, 8°50' W) and displays a warm temperate climate. With an area of around 8.6 km², this small estuary, at about 7 km from the sea, branches into 2 arms (north and south), separated by the small island Murraceira, which join again near the mouth (Fig. 1).

The two arms exhibit different hydrographic features. The deeper northern arm (4 to 8 m during high tide, tidal range of 1 to 3 m), presents shorter residence times (<1 day), constitutes the major navigation channel of the Figueira da Foz harbour, and consequently suffers regular dredging activity. The shallower southern arm (2 to 4 m during high tide, tidal range of 1 to 3 m), displays longer residence times (2 to 8 days), and it is greatly silted up, particularly in upstream areas, causing most of the freshwater discharge to flow through the northern arm (Cardoso et al., 2004; Pardal et al., 2000). Water circulation in this arm is mostly due to tidal cycles, along with small freshwater input from the Pranto River tributary, which is controlled by a sluice located at 3 km from the confluence with this arm (Marques et al., 2003), additionally, symptoms of eutrophication are visible (Cardoso et al., 2004; Marques et al., 2006, 2007a; Pastorinho et al., 2003).

Greater depth in the northern arm causes faster tide penetration which produces larger daily changes in salinity, whereas temperature shows more daily variations in the southern arm (Marques et al., 2002). Salt marshes dominated by *Spartina maritima* can be seen along the southern arm (Dolbeth et al., 2011), but most of this area is comprised of intertidal mudflats, with these being less extensive in the north arm (Martinho et al., 2007).

The Mondego estuary has experienced eutrophication since the 1980s owing to excessive nutrient-loading coming from the Pranto River, urban areas, industrial, agricultural and aquaculture activities (Cardoso et al., 2004). However, since 1996, several mitigation measures have been implemented (e.g., reduction of nutrient loading, protection of *Zostera noltii* seagrass beds, and enhancement of freshwater circulation), causing the system to show signs of gradual recovery (Cardoso et al., 2005, 2008; Marques et al., 2003; Martinho et al., 2007).

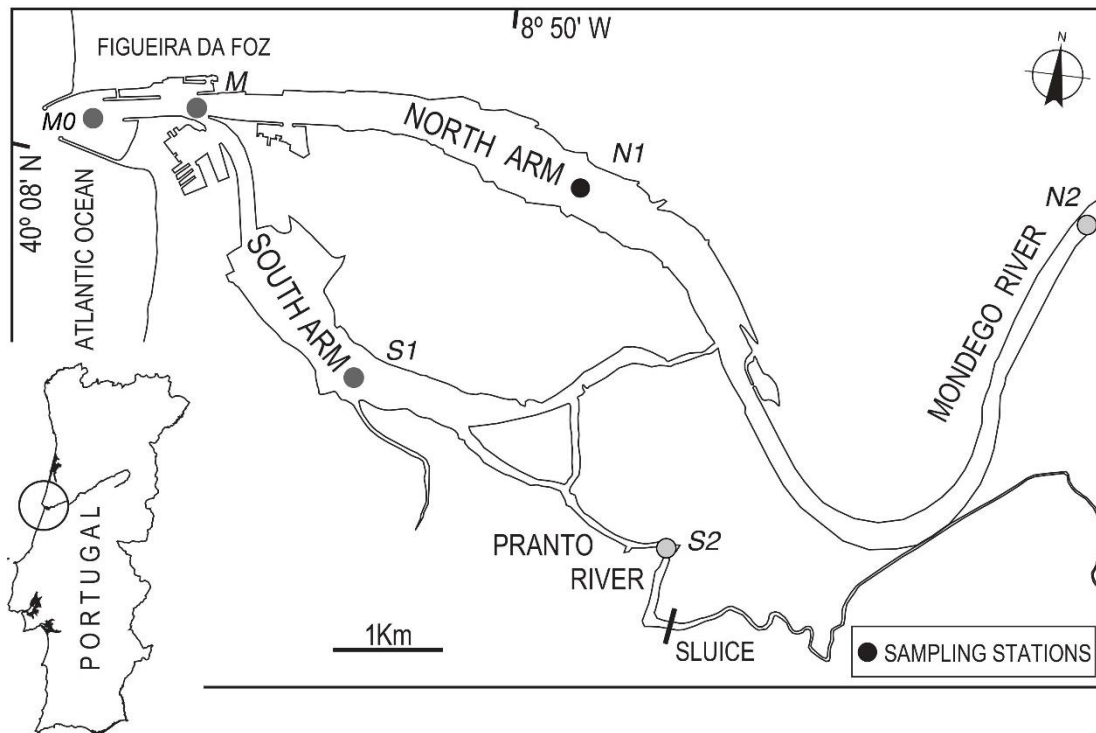


Figure 1. Map of the Mondego estuary located in western Portugal, showing the position of the six sampling stations. M0, M and S1 – downstream sampling station; N1 – middle estuary; S2 and N2 – upstream sampling stations.

2.2. Sampling and laboratory procedures

2.2.1. Sampling methodology

Sampling was carried out monthly at high tide, from January 2003 to December 2012 at 5/6 stations, one/two at the mouth of the estuary (M0 and M), and two in each arm, S1 and S2 in the south arm, and N1 and N2 in the north arm, covering the salinity gradient (Fig. 1).

Zooplankton were gathered by performing horizontal subsurface tows (Bongo net: mesh size 335 μm , mouth diameter: 0.5 m, tow speed: 2 knots), equipped with a Hydro-Bios flowmeter (volume filtered averaged 45 m^3). The resulting samples were preserved in a 4% borax-buffered formaldehyde seawater solution and returned to the laboratory for analysis. Additionally, at each site, salinity, water temperature (T, $^{\circ}\text{C}$), dissolved oxygen (DO, mg L^{-1}), and pH were measured *in situ*, using WTW Cond 3310 sensors. Alongside, at each location, water (1 L) was collected, for further analysis and turbidity (Secchi disc depth, m) was recorded.

2.2.2. Laboratory procedures

The 1 L water samples were filtered with Whatman GF/C glass fibre filters followed by Chlorophyll *a* extraction, respecting the protocol of Parsons et al. (1984), and suspended particulate matter (SPM) determination.

Zooplankton samples were washed with freshwater and transferred to 70% alcohol in identified airtight plastic bottles, for easier storage. 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 (335 μm), to remove debris. The resulting sieved contents were examined in Bogorov counting chambers under Leica M80 stereomicroscopes, and observed organisms were counted and identified to the lowest possible taxon. Samples with excessive numbers of organisms were sub-sampled as necessary by using a Folsom plankton splitter. The final data set comprised the number of individuals per taxon for the 2003-2012 period.

From the original database, the most abundant/frequent taxa were selected for biomass determination: The Copepoda *Acartia tonsa*, *Acartia clausi*, *Temora longicornis*, *Copiodiaptomus numidicus*, *Acanthocyclops trajani*, *Paracalanus parvus*, and *Clausocalanus arcuicornis*, the Cladocera *Penilia avirostris*, *Daphnia* sp., *Bosmina* sp., and *Evadne nordmanni*, Siphonophora, the Tunicata *Oikopleura dioica* and the Chaetognatha *Sagitta friderici*. 50 individuals from the required taxonomic groups were sorted from random samples (2003-2012) and placed in small previously weighed empty aluminium crucibles sited in heat resistant acrylic multiwell trays. These were afterwards put in a drying oven set to 60°C for at least 24h, as recommended (Lovegrove 1962, 1966). This assured water removal without affecting organic content (Ré et al., 2005). Subsequently, the capsules were weighed using an electronic micro-balance with an accuracy of 0.1 mg and values were annotated. At least 10 samples (with 50 organisms each) were weighed for each taxon.

Dry weight values were obtained by subtracting the initial weight of empty aluminium crucibles from the values registered after 24h at the drying oven. Afterwards, the individual weight for a given taxonomic group was determined by dividing the total dry weight of each sample by the number of individuals. Later, a biomass matrix (2003-2012) was obtained by multiplying the individual weight to the abundance matrix (2003-2012). Finally, biomass was converted into volumetric biomass (mg m^{-3}) by dividing them with the initial volumes and fractionation of samples.

Conversion to carbon weight ($\mu\text{g C}$) was done assuming that it was 40% of dry weight, according to the methods employed by Omori & Ikeda (1984) and Båmsteadt

(1986). These values represent a realistic estimate of biomass and the nutritive value of zooplankton (Ré et al., 2005).

Secondary production was determined, by using the previously obtained volumetric biomass matrix, with the following equation:

$$P = Bg \quad (1)$$

Where P is the production ($\text{mg C m}^{-3} \text{d}^{-1}$), B is biomass (mg C m^{-3}), and g is the weight-specific growth rate (d^{-1}). Copepod growth rates estimates were obtained from the temperature-dependent predictive method of Huntley & Lopez (1992), with the expression:

$$g = 0.0445e^{0.111T} \quad (2)$$

T equals temperature ($^{\circ}\text{C}$). These authors have shown that for marine copepods, temperature dependence of growth rates beats species differences. As a result, the exponential growth model presented should be applicable to produce estimates of the entire communities, regardless of species composition or size frequency. Hence, in this study, growth was estimated from adult organisms' weight for each taxonomic group and the recorded temperatures at the corresponding sampling site.

2.3. Data preparation and statistical analysis

Samples were averaged by estuarine section, downstream (M0, M, S1), middle (N1) and upstream (N2, S2), and taxa by the ecological guild (marine, estuarine and freshwater; Table 1). Copepod biomass/production was averaged by month and log-transformed ($\log_{10}(x+1)$). Then, regression on the copepod biomass/production monthly time series was performed. For that, each time series was seasonally detrended by computing a 12-month centred moving average of transformed data. Afterwards, long-term annual and seasonal patterns of copepod biomass and production were analysed based on anomalies. For each, the anomaly (A'_m) was calculated using the formula:

$$A'_m = \left(\frac{\bar{A}_m}{\bar{A}_i} \right) \quad (3)$$

Where \bar{A}_m is the transformed average for a given year/month m , and \bar{A}_i is the long-term transformed mean for calendar month i . Annual anomalies were then calculated as the average for each year (O'Brien et al., 2008).

The links between copepod biomass/production and the environmental forcing were assessed by Pearson correlation analysis. Environmental factors included local

hydrological variables (river runoff, water salinity and temperature *in situ*), regional environmental data (precipitation, sea surface temperature and sea level pressure) and the large-scale North Atlantic Oscillation (NAO) index¹. Monthly precipitation values were acquired from INAG – Instituto da Água² measured at the Soure 13 F/01G station. The freshwater discharge from the Mondego River was obtained from INAG station Açude Ponte Coimbra 12G/01AE. SST (sea surface temperature) and SLP (sea level pressure) were acquired from the International Comprehensive Ocean Atmosphere Data Set (ICOADS) online database¹. Environmental variables were normalized, and a principal component analysis was applied to local and regional variables to identify dominant patterns and extract the PC1 values of each analysis. Finally, Pearson correlation analysis between annual copepod biomass/production and environmental parameters was performed in R software (RDevelopment Core Team, 2009) using corrplot library and considering a significance level of 0.05.

¹ <http://dss.ucar.edu/pub/coads>

² <http://snirh.pt/>

3.1. Interannual environmental characterization

Interannual variations of local (river runoff, water salinity and temperature *in situ*), regional (precipitation, sea surface temperature and sea level pressure), and large-scale (NAO) environmental factors, recorded during the study period, are shown in Fig. 2. Regarding hydrological conditions, runoff (Fig. 2A) and precipitation (Fig. 2D) displayed high variability at the interannual scale. For example, in 2003 (the first year in the study), it was recorded the highest value of runoff, and one of the highest, concerning precipitation (together with 2006), later in 2004 (the following year) the lowest value for precipitation was exhibited, however, it was only in 2005 that the runoff showed the lowest value. A similar variance was observed in the period 2006/2008 and 2010/2012. Leaving 2009 as a regular year.

Salinity (Fig. 2B) followed the characteristics of the runoff and precipitation, this was expected, as it is directly influenced by them. The lowest salinity recorded was in 2006, followed by 2003 alongside 2010, and the highest value was observed in 2005, followed by 2008 and 2012. Concerning Chlorophyll *a* (Fig. 2C), 2004 exhibited the lowest values, followed by 2003, and 2008 showed the highest, followed by 2005 and 2007. In the period 2004/2005, a great rise in Chl *a* values was observed. The remainder of the years were alike, it seems that after 2008 the values stabilized.

Sea surface temperature (Fig. 2E) highest value happened in 2006, followed by 2011 alongside 2008, and the lowest values occurred in 2007 and 2009. In 2006 there was a rise in temperature until it reached the highest point in the study period, then in the next year (2007), the temperature dropped to one of the lowest values. This period was followed by similar variations in 2008/2009 and 2011/2012 (less intense). The North Atlantic Oscillation index only showed a period of great variation between 2010, with the lowest value recorded, and 2011, with the highest (Fig. 2F).

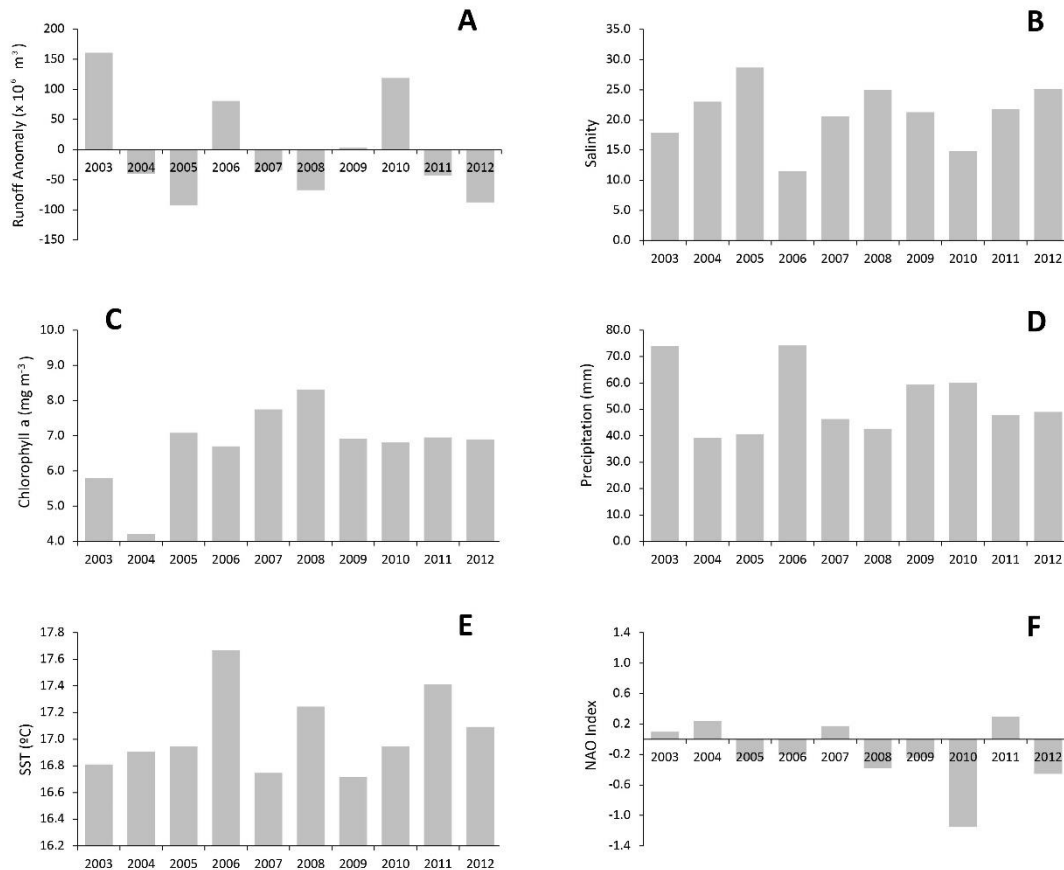


Figure 2. Interannual variation of local (A, B and C), regional (D and E) and large scale (F) environmental factors. (A) River runoff anomaly ($\times 10^6 \text{ m}^3$), (B) Estuarine water salinity, (C) Chlorophyll a (Chl a, mg m^{-3}), (D) Precipitation (mm), (E) Sea Surface Temperature (SST, $^{\circ}\text{C}$) and (F) North Atlantic Oscillation Index (NAO).

3.2. Interannual and spatial variation of zooplanktonic biomass

Interannual relative biomass proportions of the taxonomic groups constituting the community are presented in Fig. 3. Copepoda is the most representative group, reaching around 85% (the highest value) of the whole community, in 2006. Most evident variations related to this group, occurred in the period 2003/2004, with a rise in the representative percentage, followed by a drop in 2004/2005 and another rise in 2005/2006, with the value in 2005 being the lowest (Fig. 3).

Chaetognatha reached the second-highest value ($\approx 30\%$) in 2005. Most apparent variations occurred between 2004/2005, where an accentuated rise happened, followed by an even more substantial drop between 2005/2006. After this decrease, the group exhibited low values until 2010 (Fig. 3). Cladocera reached the third highest value ($\approx 25\%$) in 2010. Most noticeable variations happened between 2009/2010, where a rapid

rise occurred, and between 2010/2011, with a large decrease. This group's lowest value was exhibited in 2004 (Fig. 3).

Siphonophora and Tunicata were the least representative groups, reaching the highest values ($\approx 17.5\%$ and $\approx 2.5\%$, respectively) in 2003. For Siphonophora, most evident variations occurred between 2003/2004, with a severe decrease, and between 2009/2010, with another harsh decrease. Concerning Tunicata, which was the least representative group, values did not differ much, however, in 2003, 2008 and 2009 the group had a higher representation (Fig. 3). In the period between 2006 – 2009 predator groups (Chaetognatha, Tunicata and Siphonophora) exhibited an increase (Fig. 3).

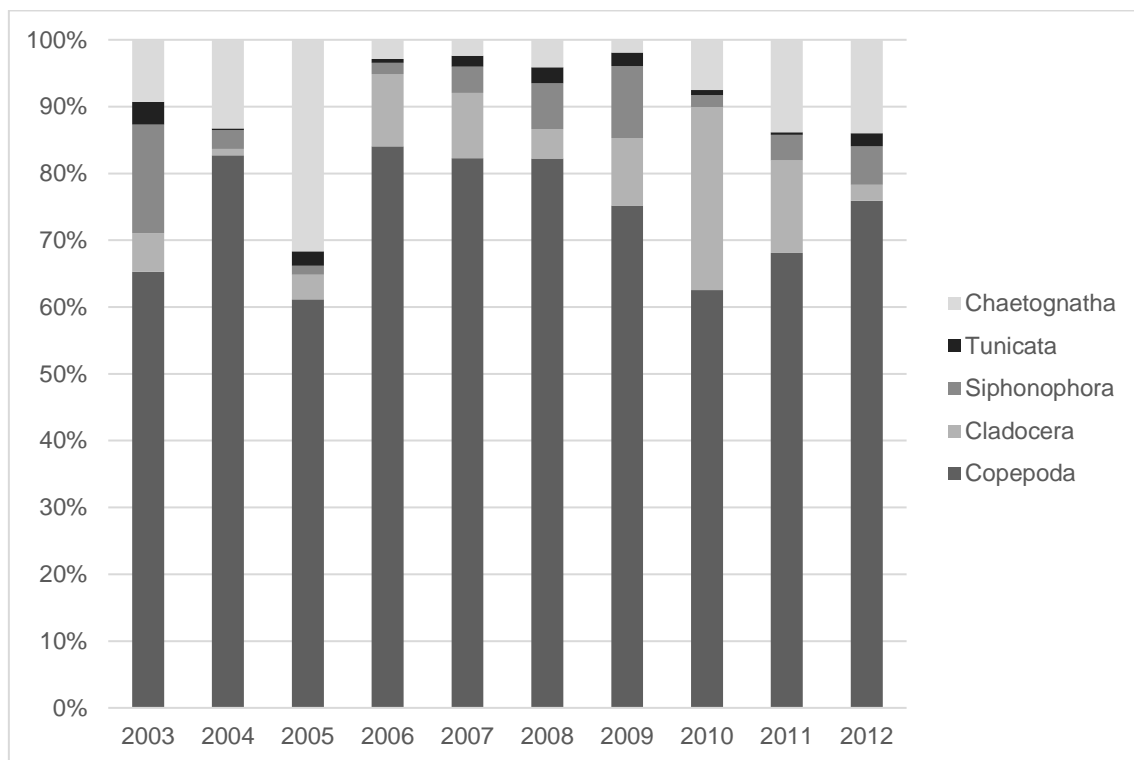


Figure 3. Relative biomass proportions, in percentage, of the taxonomic groups constituting the community.

Interannual changes in biomass of marine, estuarine, and freshwater species, for the three estuarine areas (upstream, middle, and downstream), are described in Fig. 4. The most apparent deviation occurred in 2009, where most of the communities reached a maximum in biomass (Fig. 4). Regarding the three areas, estuarine species were the most representative upstream, and marine species dominated in the middle and downstream of the estuary. Freshwater species also reached the highest values during 2009, in the middle area, almost reaching the same values as marine species (Fig. 4).

The only communities that had a different response during 2009, were estuarine species in the middle of the estuary, which exhibited a decrease, and downstream freshwater species, which only reached their peak in 2010 (Fig. 4). In general, between 2007 – 2009, occurred a decrease in freshwater communities and a simultaneous increase in more salinity tolerant species (marine and estuarine). However, between 2008/2009, some influence promoted the increase of all communities (Fig. 4). Concerning marine species, they exhibited the highest value downstream, and the lowest upstream. The upstream community displayed higher interannual variability than the others. Estuarine species, showed the highest value upstream, presenting the biomass peak of the study period. The estuarine species inhabiting the middle of the estuary displayed higher interannual variability (Fig. 4). Lastly, regarding freshwater communities, they exhibited higher interannual variability than the others. Freshwater species in the middle and upstream areas reacted similarly between 2003 – 2008. In the period 2003 – 2005 downstream freshwater species responded differently, displaying low values. Additionally, in 2009, this community exhibited a smaller increase, and in 2010, responded differently from the others inhabiting different zones, by having an increase in biomass.

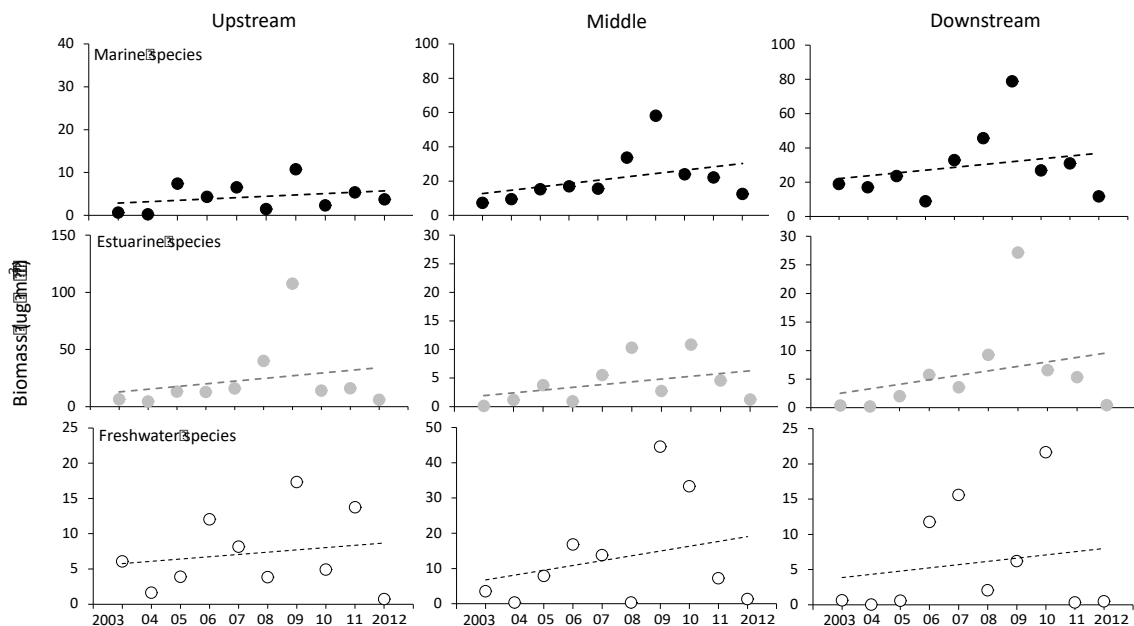


Figure 4. Changes in biomass of marine, estuarine, and freshwater species over time for the three estuarine areas (upstream, middle, and downstream). Significant linear regressions of annual means ($P < 0.05$) are shown with solid lines whereas non-significant linear trends are indicated with dashed lines. Notice the different scaling on the y-axes.

The interannual mean biomass, of the species and taxonomic groups inhabiting the three areas, is displayed in Table 1. The highest value of biomass belonged to the estuarine species *Acartia tonsa* during 2009 in upstream areas. In these areas, besides this species, *Daphnia* sp. (in 2011) and *Copidodiaptomus numidicus* (in 2006) were the most representative (Table 1). In the middle areas of the estuary, the highest values occurred in 2009 and belonged to the species *Acartia clausi* and *Copidodiaptomus numidicus* accompanied by the taxonomic group Siphonophora. Downstream areas were dominated by *Acartia clausi* with the highest value in 2009, followed by *Acartia tonsa*, also in 2009.

Table 1. Biomass ($\mu\text{g m}^{-3}$) of the various community components for the three areas (upstream, middle, and downstream), over the years.

			2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Marine species	<i>Oikopleura dioica</i>	Upper	<0.01	0.01	0.45	0.14	0.33	0.07	1.75	0.14	0.01	0.52
		Middle	0.34	0.05	0.37	0.17	1.07	1.19	2.17	0.31	0.30	0.10
		Lower	1.16	0.02	0.86	0.22	0.46	2.30	3.22	0.63	0.14	0.13
	<i>Sagitta friderici</i>	Upper	<0.01	<0.01	2.27	0.67	0.42	0.21	0.61	0.09	3.37	1.26
		Middle	1.40	2.14	10.21	1.39	0.12	2.38	2.29	2.03	8.70	1.68
		Lower	2.67	2.42	11.98	0.51	2.29	3.41	3.90	8.73	3.12	2.39
	<i>Evadne nordmani</i>	Upper	<0.01	<0.01	0.26	0.03	0.16	0.02	0.07	0.02	<0.01	<0.01
		Middle	0.01	<0.01	0.08	0.04	0.16	0.38	1.73	0.29	0.03	0.09
		Lower	0.35	<0.01	0.50	0.35	0.76	1.52	0.82	1.25	0.11	0.05
	<i>Penilia avirostris</i>	Upper	0.35	0.04	0.17	0.43	0.35	0.23	1.58	0.54	0.85	0.04
		Middle	0.15	<0.01	0.16	0.55	1.78	0.03	2.77	5.82	2.33	0.03
		Lower	0.13	<0.01	0.06	0.26	0.97	0.03	0.73	2.46	0.04	0.02
	<i>Acartia clausi</i>	Upper	0.01	0.08	1.81	1.66	1.60	0.21	3.28	0.82	0.07	0.31
		Middle	1.44	5.35	2.70	10.52	8.17	19.70	21.68	8.73	5.37	5.83
		Lower	6.46	10.90	5.96	5.26	17.95	22.10	41.85	9.87	17.20	5.59
	<i>Clausocalanus arcuicornis</i>	Upper	0.00	0.01	0.14	0.07	1.54	0.04	0.32	0.51	0.01	0.06
		Middle	0.04	0.03	0.17	0.33	2.62	2.83	1.68	0.89	0.37	0.36
		Lower	0.38	0.15	0.64	0.63	3.85	4.49	6.87	0.68	1.71	0.39
	<i>Paracalanus parvus</i>	Upper	0.01	0.01	0.17	0.19	0.03	0.01	0.34	0.09	0.53	0.53
		Middle	0.06	0.50	0.07	0.11	0.05	0.30	1.53	3.81	2.75	1.74
		Lower	0.35	0.68	0.03	0.37	0.04	0.24	2.80	1.66	5.78	1.14
	<i>Temora longicornis</i>	Upper	0.06	0.07	2.02	0.93	0.64	0.32	0.60	0.11	0.50	0.84
		Middle	1.17	1.23	1.30	2.91	1.11	2.64	3.02	0.27	1.80	1.37
		Lower	3.15	2.00	2.74	0.85	3.77	6.18	4.09	0.80	2.86	1.27
Siphonophora	Upper	0.22	0.00	0.13	0.21	1.45	0.37	2.19	0.03	0.04	0.14	
	Middle	2.63	0.14	0.15	0.86	0.45	4.21	21.31	1.77	0.50	1.30	
	Lower	4.23	0.84	0.74	0.45	2.71	5.41	14.47	0.77	3.67	0.74	
Freshwater species	<i>Acanthocyclops trajani</i>	Upper	0.78	0.10	0.26	0.95	0.79	0.52	3.52	1.20	1.90	0.10
		Middle	0.34	0.01	0.33	1.22	3.96	0.06	6.16	12.95	5.18	0.07
		Lower	0.28	0.00	0.13	0.59	2.15	0.07	1.63	5.48	0.08	0.04
	<i>Bosmina</i> sp.	Upper	1.10	0.05	0.18	0.24	2.27	0.07	0.68	0.46	0.18	0.04
		Middle	0.34	0.01	0.04	0.20	0.68	0.05	0.44	1.53	0.01	0.00
		Lower	0.13	<0.01	<0.01	0.15	0.20	0.14	0.04	0.73	0.01	0.00
	<i>Daphnia</i> sp.	Upper	0.00	0.18	0.30	1.26	0.66	1.97	8.44	2.41	10.43	0.06
		Middle	0.00	0.03	0.91	2.99	2.04	0.22	17.03	13.35	0.98	0.40
		Lower	0.00	0.01	0.20	3.23	1.43	1.83	1.31	10.64	0.25	0.19
	<i>Copidodiaptomus numidicus</i>	Upper	4.18	1.28	3.15	9.58	4.43	1.27	4.67	0.82	1.21	0.52
		Middle	2.88	0.30	6.65	12.43	7.10	0.05	20.94	5.49	1.05	0.90
		Lower	0.24	0.03	0.27	7.79	11.81	0.04	3.21	4.79	0.03	0.28
Estuarine species	<i>Acartia tonsa</i>	Upper	6.25	4.39	13.01	12.71	15.71	39.87	107.39	13.87	15.89	5.89
		Middle	0.10	1.13	3.72	0.90	5.48	10.27	2.70	10.82	4.55	1.22
		Lower	0.38	0.21	2.01	5.74	3.60	9.24	27.15	6.57	5.98	0.46

3.3. Long-term patterns of Copepoda biomass and production

Biomass and production interannual variations, of the different Copepoda communities, are shown in Fig. 5. Marine and estuarine communities displayed a significant increasing trend for both biomass and production. The freshwater community did not exhibit a significant trend, however, it seems to maintain a stable state (Fig. 5).

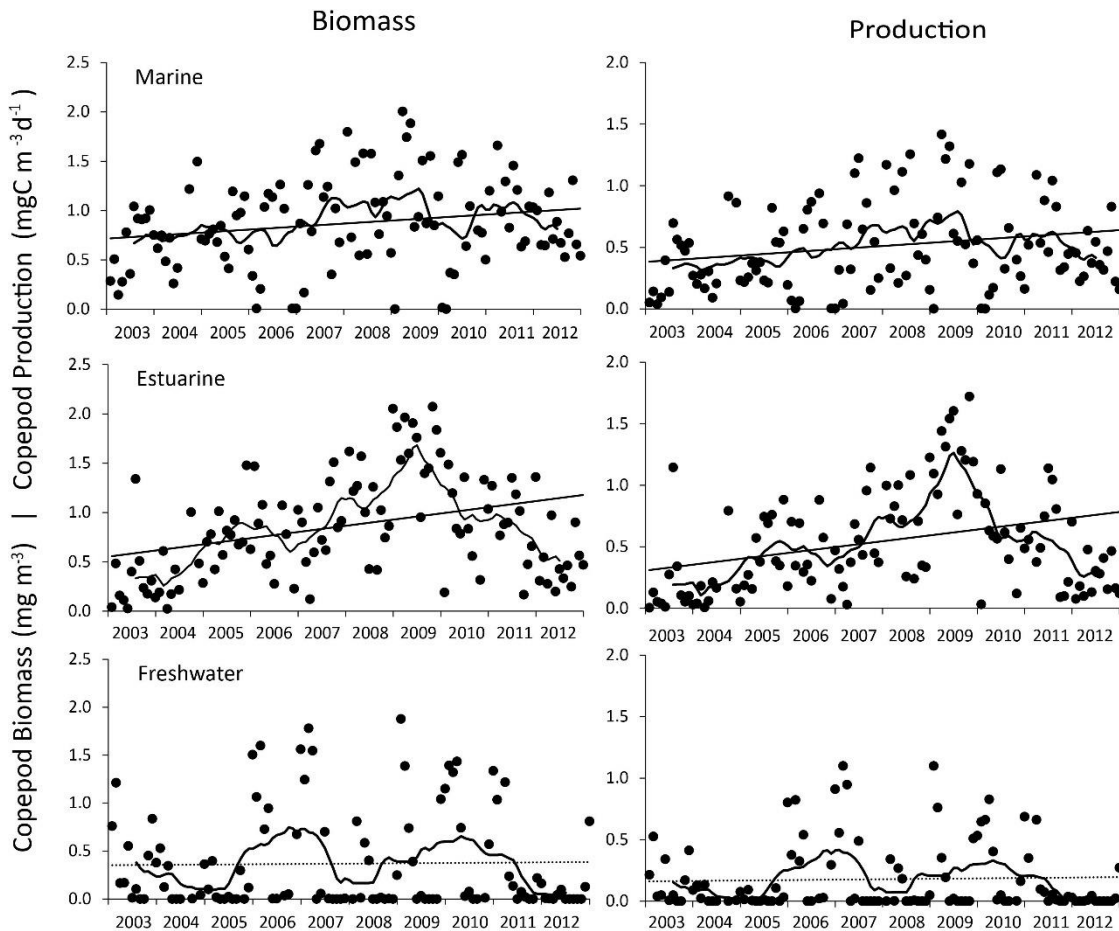


Figure 5. Long-term variation of copepod biomass and production. Black dots represent monthly values; solid black curve represents the 12-month centred moving average (detrend) and significant linear regression ($P < 0.05$) are shown with solid lines. Notice the different scaling on the y-axes.

Monthly biomass and production variations of the different Copepoda groups are shown in Fig. 6. No clear seasonal trend for marine and estuarine species, concerning biomass and production, however, both groups seem to be promoted in the summer months. For freshwater species, the reverse is observed (Fig. 6).

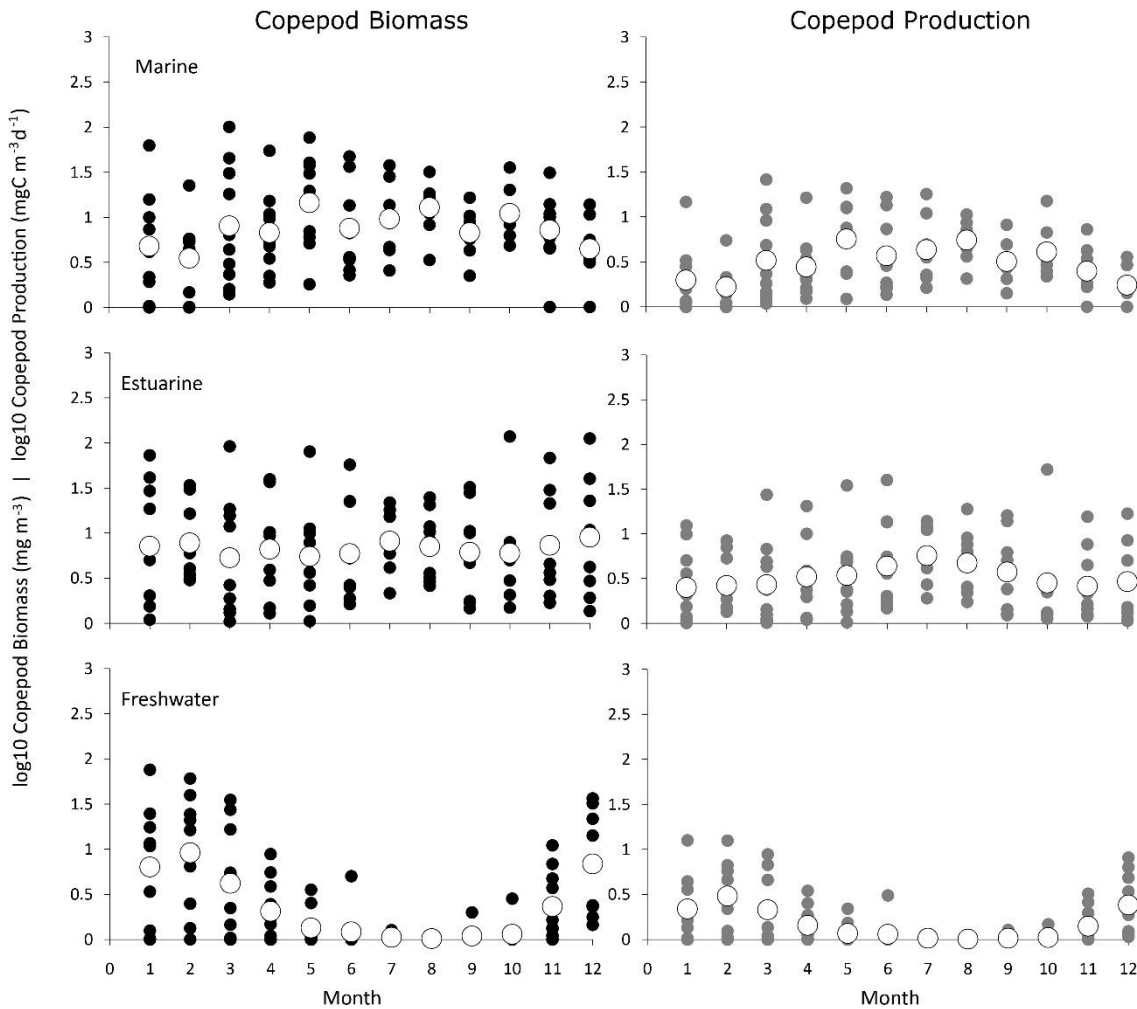


Figure 6. Copepod biomass and production monthly variation over the 2003 – 2012 period. Filled in dots represent the monthly values for each year; empty dots represent the monthly mean using the annual monthly values (filled in dots). x-axes shows the months of the year (1 – 12 signifies January – December).

Biomass and production annual anomalies are shown in Fig. 7, all the communities exhibited strong interannual variability. Copepod community presented pronounced low biomass and production in 2004 along with 2012, while in 2009 values were higher.

Marine copepods exhibited a successive rise in biomass and production values between 2007/2009. A similar increase by estuarine copepods was also experienced in this period, however, the rise was more abrupt, reaching the highest positive anomaly observed in the study. In opposite, during 2008, freshwater species reacted negatively. Freshwater copepods responded differently from the others, reaching the highest values of biomass and production in the periods 2006/2007 and 2009/2010, yet having greater biomass in 2006 and 2010, additionally in 2003 while the other communities had a negative response, this community responded positively, having an increase in biomass.

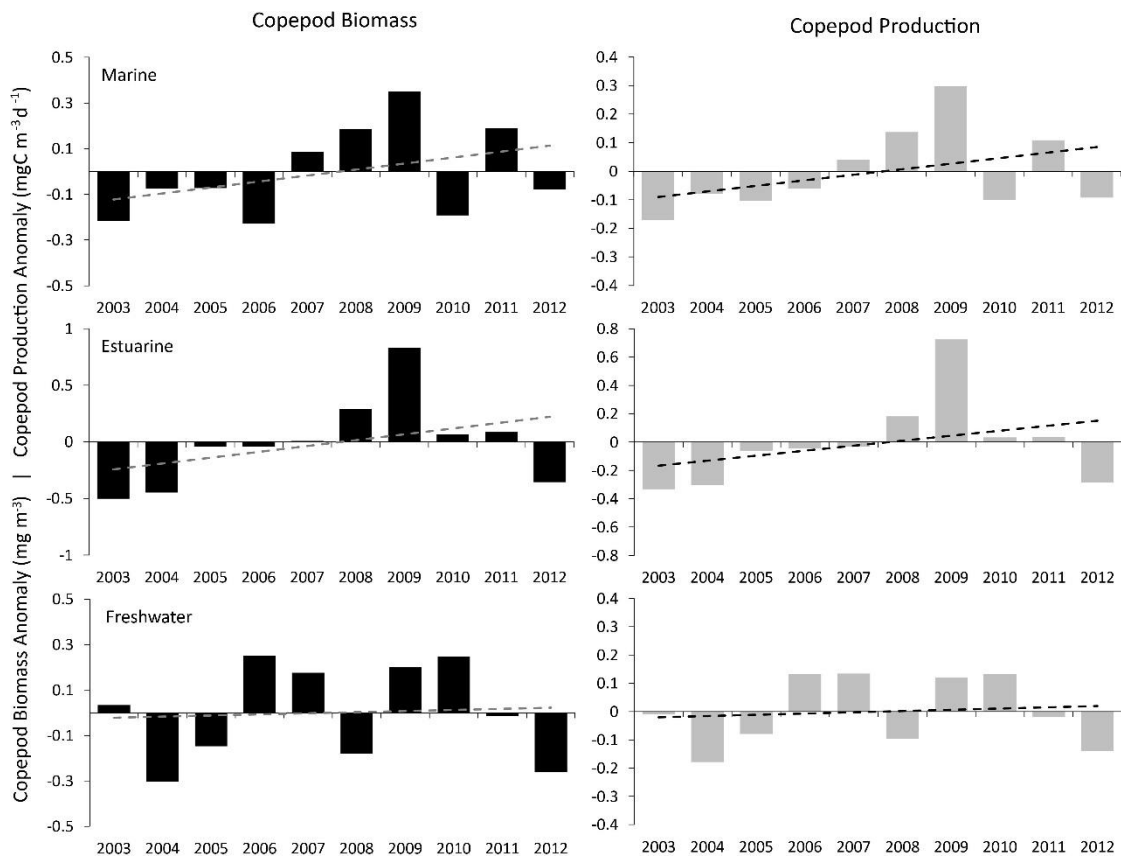


Figure 7. Annual variation (anomalies) of copepod biomass and production. Notice the different scaling on the y-axes.

3.4. Local, regional, and large-scale environmental influence on Copepoda biomass and production

Interannual variation of local (river runoff, water salinity and temperature *in situ*), regional (precipitation, sea surface temperature and sea level pressure), and large-scale (NAO) environmental factors as anomalies, is shown in Fig. 8. Salinity (Fig. 8A) reached a maximum in 2005, and this value was followed by a great decline in 2006. Chlorophyll *a* (Fig. 8B) recorded its lowest values in 2004 while its highest value happened in 2008. PC1 Regional (Fig. 8C) exhibited its highest values in 2009 2003 and 2007, additionally, the lowest values were observed in 2011. PC1 Local (Fig. 8D) displayed high interannual variability, with the lowest values occurring in 2003, 2010 and 2006, furthermore, the highest values happened in 2005, 2008 and 2012. Predators' biomass (Fig. 8E) was promoted in 2008 and 2009, while below-average abundances occurred in 2006 and 2004. NAO index (Fig.8F) was mainly positive in the 2003/2007 period and negative in the 2008/2012 period, with a severe decrease in 2010.

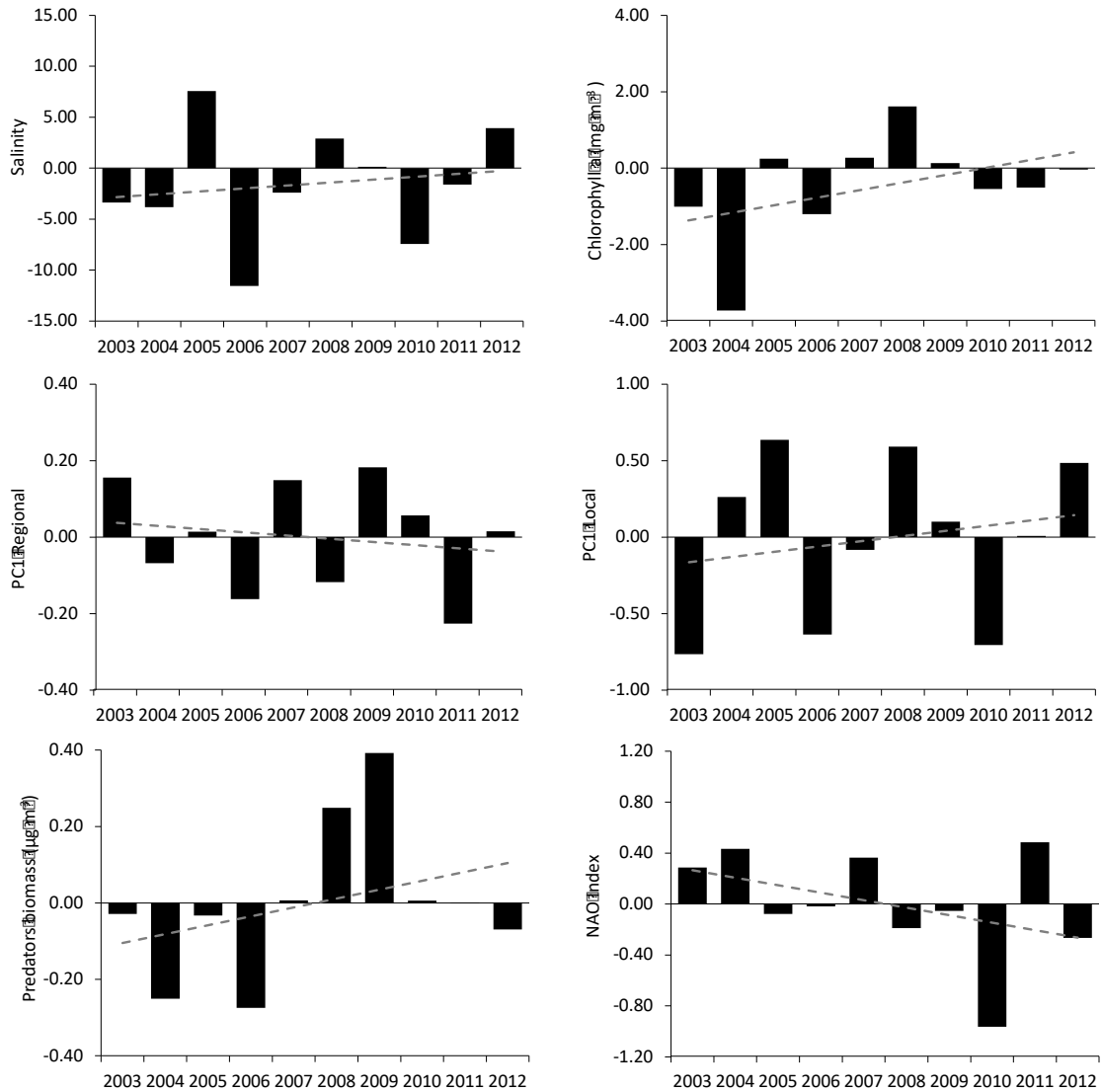


Figure 8. Long-term variation of environmental factors, local (PC1 56.9%), regional (PC1 52.6%) and large scale (North Atlantic Oscillation Index) climate observed (as anomalies). Significant linear regressions of annual means ($P < 0.05$) are shown with solid lines whereas non-significant linear trends are indicated with dashed lines.

Analysis of correlation between the environmental and copepod biological factors are displayed in Fig. 9. Marine and estuarine copepods' biomass and production exhibited positive correlations with planktonic predators. For freshwater copepods, biomass presented a negative correlation with local variables (PC1 Local) and Salinity, while production was only negatively correlated with Salinity.

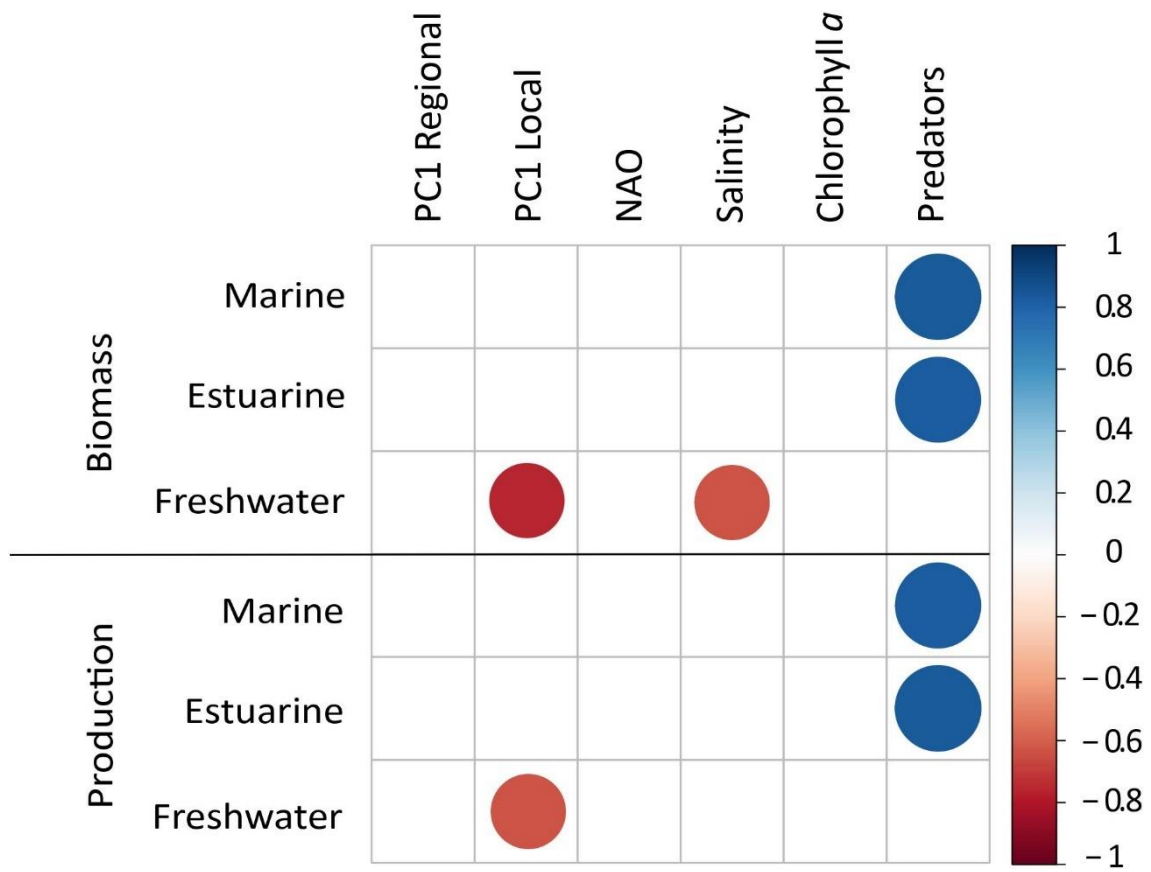


Figure 9. Results of the Correlation analysis.

4.1 Interannual and spatial variation of zooplanktonic biomass

Copepoda was the most representative group (Fig. 3), which is in accordance with previous studies of the zooplankton community in the Mondego (Gonçalves et al., 2015; Marques et al., 2006), and in other geographical areas. Seeing that copepods usually contribute to the majority of zooplankton biomass and species diversity in estuaries (Arashkevich et al., 2002; Leandro et al., 2007; Uye & Liang, 1998). Accordingly, the most representative species was the estuarine *A. tonsa*, reaching the highest value of biomass, during 2009 in upstream areas. This dominance of *A. tonsa* is typical of estuarine environments located on the Atlantic European coast (David et al., 2005; Hoffmeyer, 2004; Mouny & Dauvin, 2002; Tackx et al., 2004). Additionally, the marine species *A. clausi* dominated downstream zones. *A. clausi* is a marine neritic species (Marques, et al., 2007a) and usually occupies the outer part of the estuary (Azeiteiro et al., 1999; Marques et al., 2006). Lastly, in the middle zone of the estuary, *A. clausi* and the freshwater *Copiodiaptomus numidicus* alongside the taxonomic group Siphonophora, reached the highest values with similar amounts.

Siphonophora reached a particularly high value in 2003. This was previously observed in the area (D'Ambrosio et al., 2016; Primo et al., 2009, 2012), with high abundances of *Muggiaea atlantica*. According to Blackett et al. (2014), it seems that while Siphonophores are generally considered strictly oceanic and intolerant of low salinity waters (Mackie et al., 1987), the neritic *M. atlantica* can exploit both low salinity (Kitamura et al., 2003; Marques et al., 2008; Primo et al., 2012) and high salinity waters (Licandro et al., 2012). In 2005, a similar growth happened to Chaetognatha, probably caused by the higher marine intrusion triggered by lower runoff years. Lastly, Cladocera in 2010, reached a maximum value, prompted by a rise in freshwater species (especially *Daphnia* sp.) following increased values of precipitation and runoff.

It is noteworthy to refer that gelatinous species (Chaetognatha, Siphonophora and Tunicata), due to their low dry weight, have a small contribution to biomass, nevertheless, their total predatory impact on the zooplankton community is possibly important since these carnivores have high grazing rates (Marshallonis & Pinckney, 2008; Purcell et al., 1994), they can significantly affect plankton species occurrence and size composition when numerous.

During the study period, patterns of the spatial distribution of species biomass showed that marine species established themselves in downstream and middle

estuarine areas, reaching higher levels downstream. This occurred because of the higher marine intrusion in lower areas of the estuary, which intensified in dry years as freshwater flux reached minimum values. Water circulation in these zones (downstream) is predominantly dependent on the tides (Marques et al., 2003; Pardal et al., 2000), and communities in these areas are favoured by seawater penetration. Additionally, according to previous studies (Marques et al., 2007), these communities tend to be similar in other European estuarine ecosystems (Leandro et al., 2006; Mouny & Dauvin, 2002).

Estuarine species (*A. tonsa*) were established upstream, as they are characteristic of brackish waters (Cervetto et al., 1999; Escaravage & Soetaert, 1995). *A. tonsa* is a brackish water, euryhaline and eurythermic species (Azeiteiro et al., 2005), and usually dominates the inner areas of the southern branch (Marques et al., 2006, 2007a).

Finally, freshwater species established themselves in middle areas, only being slightly outnumbered by marine ones. The middle zone comprises a transition area simultaneously influenced by tides and, in years of high precipitation, by the freshwater flow (Marques et al., 2007). This area is mainly characterized by marine species, with estuarine and freshwater taxa also frequently observed, especially during increased river discharge (Marques et al., 2007). The spatial distribution of the marine assemblages in this region is also related to the level of seawater intrusion (Marques et al., 2007).

The year 2009 was an unusually high year regarding biomass values for all taxa. According to results given by Marques et al. (2018), the Upwelling Index (UI) rose after 2007 and reached a maximum in 2008, attaining high values in 2009. Higher upwelling in the coast, leads to greater nutrient input from deep waters, enhancing primary production thus improving environmental conditions for zooplankton growth (Marques et al., 2018). This may contribute to the unusually high zooplanktonic biomass observed this year (Fig. 4).

While a global tendency of community increase is observed, no significant trends were detected. Similarly, at the scale of the Mediterranean, no zooplankton trends were identified in previous studies (Berline et al., 2012), and this lack of significant zooplankton trends can be attributed to the relative short time-series (10 years) used, not being long enough to reveal interannual variability associated with a change in climate indices (Mackas & Beaugrand, 2010; O'Brien et al., 2013), or it may be a result of the high spatiotemporal variability of the system (Berline et al., 2012), especially if the study area has suffered successive extreme events (e.g., droughts and floods) (O'Brien et al., 2013). Further possible reasons could be that the local community displays resilience

against the ongoing climate forcing in the area (Mazzocchi et al., 2012), or species are being gradually replaced by others while maintaining total zooplankton biomass values (seen in previous works) (Gonçalves et al., 2015; Marques et al., 2006; Primo et al., 2009).

4.2 Long-term patterns of Copepoda biomass and production

Regarding seasonality, freshwater copepods were the only community to experience a seasonal pattern, reaching higher values during winter. This period is associated with higher freshwater inflow along with lower salinities, therefore, allowing the establishment of this community. The opposite is observed during summer, where a higher marine intrusion is triggered.

Salinity variability in estuaries is linked to the advective properties of freshwater discharge, and plays a critical role in communities, affecting species' spatiotemporal variability (at several scales) (Fernández-Delgado et al., 2007; Licandro & Ibañez, 2000). In the Mondego estuary, river flow variation and associated changes in the salinity gradient have a strong impact on the seasonal and spatial distribution of zooplankton species (Primo et al., 2015). The strong influence of advection on these species was also observed in previous studies (Marques et al., 2006; Primo et al., 2009, 2011).

The lack of cyclic variation regarding marine copepods may be related to species replacement when they are seasonally substituted by each other yet maintaining average biomass (Gonçalves et al., 2015). The interaction between tidal currents and river flow potentially masks the seasonality of zooplankton by introducing a superimposed variability, that is reflected by the complex response of zooplankton to the biological and environmental conditions (D'Alcalà et al., 2004). In the western Mediterranean basin, the range of zooplankton biomass and occurrence is not always well defined (Gonçalves et al., 2015), unlike neritic and oceanic regions (Calbet et al., 2001; Fernández de Puelles et al., 2003; Gilabert, 2001), in estuaries there is a lack of seasonality in zooplankton abundance throughout the years (Marques et al., 2014).

During drought periods reduction in zooplankton seasonality and salinity-associated differences between upstream and downstream communities were observed (Marques et al., 2006; Primo et al., 2009).

Estuarine copepods did not differ seasonally, according to Marques et al. (2006), the population of *A. tonsa* found in the southern arm, is probably well-established, and have adapted their physiological tolerances (Cervetto et al., 1999), resulting in low

seasonal variability. Their eurythermic behaviour explains why *A. tonsa* copepods are found in the plankton year-round (Azeiteiro et al., 2005).

Generally, marine copepod biomass and production were higher in years with low precipitation/runoff (2008, 2011), while the opposite was observed for freshwater copepods (2006, 2010). As previously mentioned, the year 2009 displayed a peak in biomass and production, which could have been caused by an intensified upwelling. Over the analysed period, marine and estuarine biomass and production presented increasing trends in both monthly and annual average, however, only the monthly average showed a significant long-term trend. This significant long-term trend of increase was probably caused by the progressive alteration induced by climate change (combined with anthropogenic forcing) in this area. Change in the properties of the estuary over the years, promoting higher dominance of these communities (increased mean temperature, salinity, etc.). During the last century, coastal waters have warmed leading to profound costs for the dynamic regime of coastal ecosystems (Goberville et al., 2010; Scavia et al., 2002). Previous studies indicated a link between temperature and changes in zooplankton, with water temperature presenting a positive correlation with all of the main marine groups in the Mondego estuary (D'Ambrosio et al., 2016; Marques et al., 2014). Temperature strongly affects vital physiological rates such as respiration and excretion (Gaudy et al., 2000; Roddie et al., 1984). And it's capable of modifying life-history traits of zooplanktonic organisms, such as copepods, through its influence on egg production, growth, development, and mortality rates (Halsband-Lenk et al., 2002; Hirst & Kiørboe, 2002; Leandro et al., 2006). Consequently, affecting phenology and dormancy cycles of certain planktonic species (Edwards & Richardson, 2004).

A significant long-term increase in mesozooplankton biomass has also been documented in the North Atlantic subtropical gyre at the Bermuda Atlantic Time series Study (BATS) site (Steinberg et al., 2012), and in the North Pacific subtropical gyre at the HOT station ALOHA (Hannides et al., 2009; Sheridan & Landry, 2004).

According to Steinberg et al. (2012) this increase in zooplankton biomass was most likely driven through bottom-up control by smaller phytoplankton, which increased in biomass and production, climbing up the microbial food web into mesozooplankton. Furthermore, Sheridan & Landry (2004) proposed that the increase in zooplankton biomass was linked to the increasing role of input of new nutrients via N fixation, done by smaller phytoplankton (Dore et al., 2002; Karl, 1999). This was possibly caused by enhanced stratification, which led to increases in ecosystem productivity.

Relationships with environmental factors showed a strong negative influence of river runoff, salinity, and local temperature (PC1 Local; salinity) on the biomass and production of freshwater copepod while marine and estuarine copepods' biomass and production exhibited positive correlations with planktonic predators (Fig. 9). Freshwater copepods are less tolerant to salinity changes and their presence in the Mondego estuary are strongly linked to the freshwater river flow. In periods of enhanced marine intrusion, the higher osmotic stress and pressure benefits more salinity tolerant species (through predation and competition). On the other hand, the positive relationship between marine and estuarine copepods' biomass/production and predators' abundance is related to their similar salinity preferences and indicates the absence of strong predation pressure. A higher prevalence of marine copepods in the Mondego estuary may lead to shifts in the community structure inducing changes at upper trophic levels, including resident fish and commercially important nursery species.

Biomass and production fluctuated very similarly, which is expected, as production was determined using biomass and the temperature-dependent growth rate. However, the determination of production is useful to have quantifiable values for the functioning of the copepod community. The quantification of ecosystem functioning is complex, involving chemical, physical, and biological processes (e.g., decomposition, nutrient cycling, production) (Dolbeth et al., 2015). Therefore, the use of secondary production, which is a process that links population characteristics (e.g., biomass, growth rate, survivorship, and development time) to several ecosystem-level processes (Dolbeth et al., 2012), seems warranted.

Secondary production is a comprehensive measure of success for a population since it is a combination of several variables that themselves have been considered measures of fitness (e.g., density, biomass, individual growth rate, fecundity, survivorship, body size and life span) (Benke, 1993). As stated earlier, by combining secondary production with long-term datasets, the understanding of ecosystem functioning and integrity is upgraded, delivering a broader overview of the possible impacts (Dolbeth et al., 2007, 2011; Pranovi et al., 2008), for which the uses of density and/or biomass fail to provide (Dolbeth et al., 2012).

CHAPTER 5 – FINAL REMARKS

Biomass and secondary production of marine and estuarine copepods showed an increasing trend in the Mondego estuary over the study period. This seems to be related with hydroclimate changes that fostered periods of increased temperature and salinity, mainly caused by lower river freshwater inflow and consequent higher marine intrusion of seawater. Overall, inter-annual variation of the copepod community was related to the existence of extreme events, mainly droughts, which are getting increasingly common. In the Mondego estuary these extreme events have a strong influence on the zooplanktonic community, lessening significant long-term trends.

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