



Universidade de Aveiro

Departamento de Biologia

2018

**Nuno Henrique
Ferraz da Silva
Dolgner**

**Tunicata and chaetognaths
populations, their responses to
varying environmental factors in a
coastal system of Iberian Peninsula**

**Tunicados e chaetognatas, as suas
respostas aos fatores ambientais de
um sistema costeiro da Península
Ibérica**

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Dissertação apresentada à Universidade de Aveiro, para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha, realizada sob a orientação científica do Doutor Ulisses Manuel de Miranda Azeiteiro, Professor Associado com Agregação no Departamento de Biologia da Universidade de Aveiro, e coorientação da Doutora Sónia Cotrim Marques, Investigadora Pós-Doc. no Instituto Português do Mar e da Atmosfera.

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keywords Climate change; Gelatinous zooplankton; Chaetognatha; Tunicata; *Fritillaria borealis*, *Oikopleura dioica*, *Parasagitta friderici*, Environmental factors; Mondego estuary;

abstract Nowadays, the evidences relating the impacts of climate change in gelatinous zooplankton communities are recurrent, and it must be considered due to their key role in marine pelagic food webs. This study aims to investigate the latent influence of the environmental variability on interannual abundance changes of two less-talked gelatinous zooplankton groups, which were Tunicata and Chaetognatha in the Mondego estuary (Portugal), over the period 2003-2012. In this study was possible to find a correlation between environmental factors and species abundance exhibiting marked interannual variability, particularly, after drought years. In addition, higher abundances were found in the mouth and middle stations, close to the entrance of the estuary. Our results also showed several resemblances with the upshots reported in the Indian Ocean and/or in California (USA), that contributed to complement the baseline data, carrying important information about these holoplanktonic groups and the way that environmental factors affects their variability in the Mondego estuary.

palavras-chave

Alterações climáticas; Zooplâncton gelatinoso; Quetognata; Tunicata; *Fritillaria borealis*; *Oikopleura dioica*; *Parasagitta friderici*; Fatores ambientais; Estuário do Mondego;

resumo

Hoje em dia, os estudos que relacionam os impactos das alterações climáticas com as comunidades de zooplâncton gelatinoso são recorrentes, e não devem ser ignoradas devido ao seu papel fundamental nas cadeias alimentares pelágicas dos ecossistemas marinhos. Este estudo tem como objetivo investigar a influência latente da variabilidade ambiental sobre as alterações na abundância interanual de dois grupos menos estudados de zooplâncton gelatinoso, tunicados e quetognatas, no estuário do Mondego (Portugal) durante o período de 2003-2012. Neste estudo, foi possível encontrar uma correlação entre os fatores ambientais e a abundância das espécies, mostrando uma variabilidade interanual acentuada, particularmente após os anos de seca. Os organismos apresentaram maior abundância nas estações da embocadura e intermédias, perto da entrada do estuário. Os nossos resultados também mostraram várias semelhanças com os resultados relatados no Oceano Índico e/ou na Califórnia (EUA), contribuindo assim para complementar os dados já existentes, trazendo informações importantes sobre estes grupos holoplanctónicos e como os fatores ambientais afetam a sua variabilidade no estuário do Mondego.

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1. INTRODUCTION

Currently, the threats affecting marine ecosystems are vast and the impact of global climate change added to anthropogenic pressure leads to negative consequences on all marine environments (Halpern et al. 2008; Brierley and Kingsford 2009; Doney et al. 2012). These changes have a serious impact worldwide in the marine ecosystems (Brierley and Kingsford 2009; Drinkwater et al. 2010), as in the case of estuaries (Marques et al. 2018), through modifications in the physical environment, affecting directly the structure and function of marine plankton, and other levels of marine food webs (Hays et al. 2005; Bucklin et al. 2010; Molinero et al. 2013). Since the 1960s several planktonic communities altered their latitudinal distribution in consequence of the rising ocean temperatures (Beaugrand et al. 2002). Therefore the understanding of the interaction between climate and zooplankton is fundamental (Primo et al. 2012), mainly due to its pivotal role, as a secondary producer, in energy transfer between primary producers and further trophic levels (D'Ambrosio et al. 2016). Frequently, zooplankton communities are considered more sensitive indicators of marine changes than climate variables, owing to their highly sensitive capability to amplify subtle changes of environmental conditions (Taylor et al. 2002; Hays et al. 2005). Recent investigations, in the Atlantic Iberian Coast, showed that multiple nursery areas are exposed and linked to hydrographic variations, impacting pelagic communities (e.g. Marques et al. 2007; Dolbeth et al. 2010; Primo et al. 2011). In the Mondego Estuary, located in the western Iberian coast, these impacts appear to increase the abundance and predation pressure of gelatinous zooplankton (e.g. Siphonophorae and Hydrozoa) upon other zooplanktonic communities like cladocerans and copepods (D'Ambrosio et al. 2016; Marques et al. 2018).

The purpose of this research was to study the interannual and seasonal changes of three holoplanktonic species in the Mondego estuary - *Parasagitta friderici* (phylum Chaetognatha); *Fritillaria borealis* and *Oikopleura dioica* (subphylum Tunicata, class Appendicularia), over a 10-year period (2003-2012), in regard to the interannual relationship between natural environmental factors and zooplanktonic abundances. This work relies on the study of population dynamics of two gelatinous zooplankton groups poorly studied, chaetognaths and appendicularians which have plankton predation behaviour (Hickman et

al. 2008) and a filter-feeder (Aravena and Palma 2002; Flood 2003) able to survive at low nutritional environments, respectively (Sommer et al. 2000; Lobón et al. 2011).

This research will be the first study in the North Atlantic assessing exclusively the dynamics of these two groups of gelatinous zooplankton and will be an addition to the entire repertoire already published in this area, which will help to increase the knowledge and perception of climate-changing pressure on zooplankton populations.

2. STATE OF THE ART

Some preliminary work on chaetognaths and appendicularians were carried out in southern Europe, such as in the Bay of Biscay (Spain) (Intxausti et al. 2012) or in the NW Mediterranean waters (Calbet 2001; Molinero et al. 2008). Also, in other locations, like the Indian Ocean, changes in chaetognaths communities have been noticed by Kusum et al. (2014), or in the California Current (North-eastern Pacific) where the appendicularians increased their abundances and body size (Lavaniegos and Ohman 2003). However, the abundance of chaetognaths showed a different trend, decreasing in that time series (from 1978 to 2009) while increasing in the North-western Pacific region (Kang and Ohman 2014).

Additionally, changes in appendicularians and/or chaetognaths were found in some European estuaries caused by the invasion of alien species (e.g. *Mnemiopsis leidyi*, Ctenophora) such as in the Black Sea or induced by anthropogenic hydrological stressors (Shiganova 2005; Delpy et al. 2012).

In the Mondego estuary, several studies have been carried out, supported by a long-term database, to understand dynamic relationships between marine and estuarine communities and climate variability, as well as ecological and hydrological features in this area. Primo et al. (2012) brought in a significant contribution when they figured out the connection between jellyfish blooms and environmental factors, realising that temperature was a key factor as well as salinity. The prevalence of oceanic water in the estuary enhanced the abundance of marine organisms (e.g. siphonophores), and chlorophyll-*a* (Chl. *a*) concentration. The runoff was also associated with their interannual variability. In the end, the increase of water temperatures has an essential and complex role in estuarine food webs, regulating “top-

down” or “bottom-up” modifications (Frank et al. 2005; Shiganova 2005; Molinero et al. 2008).

Marques et al. (2014) added a new survey comparing zooplankton variability between drought and regular years. Considering that an irregular distribution pattern of precipitation was observed in the region, being vulnerable to droughts, the understanding between the altered hydrological regime and biological effects in the ecosystem was certainly relevant (Lehner et al. 2006). In this study, they focus on two significant drought periods in the Mondego estuary (2004-2005 and 2007-2008), as previously reported (e.g. Marques et al. 2007). However, they pointed out an intense freshwater influence period in 2010, showing that the ecology and hydrology of shallow estuaries, like the Mondego, are deeply influenced by the river flow. Even though, it was assumed that the zooplanktonic changes in response to climate variability “will affect the temporal and spatial association between species interaction of several trophic levels” (Marques et al. 2014).

Primo et al. (2015) made a synoptic climatological approach in the Mondego estuary, classifying a nine-year period as dry, average and wet conditions. This approach showed the response of zooplanktonic communities upon climate variability, even in a short period. Throughout this period significant results were detected for some marine species such as copepods and gelatinous zooplanktonic groups (e.g. siphonophores and hydromedusae), which appeared to have increased their affluence during dry periods in the estuary. Once again, both salinity and river flow variability were a significant factor leading the response of zooplanktonic organisms, which affects their spatial variability and distribution inside the Mondego estuary. This kind of approach, as synoptic climatology, is a sharp way to understand the result of climate-changing upon pelagic communities considering local scale.

Subsequently, D’Ambrosio et al. (2016) explored the connection between gelatinous carnivore zooplankton abundance and climate-driven hydrographic variations, in the Mondego estuary. Since the widespread alarming outbreaks of gelatinous plankton have been rising in several coastal waters, simultaneously the concerns about these organisms raised too, as they can change entire food webs by modifying ecosystem dynamics (Lucas et al. 2014). According to D’Ambrosio et al. (2016) results, a significant relationship between climate variability and the abundance of gelatinous carnivore zooplankton was verified (as well as their distribution in the estuary). For instance, that relationship was

improved after 2007 with the increasing activity of upwelling and local hydrology conditions. Simultaneously, local winds and precipitation raised by North Atlantic Oscillation (NAO) variations, increased the coastal upwelling activity. These relationships lean to enhance species richness and abundance of gelatinous carnivores in the estuary, and so the dynamic and structure of the marine communities could be altered (D'Ambrosio et al. 2016). Additionally, D'Ambrosio et al. (2016) mentioned that gelatinous zooplanktonic organism produced an abundance increment positively correlated with local temperatures. Because of hydroclimate forcing strength, the abundance and diversity of gelatinous zooplankton community changed, presenting a visible phenological adjustment (Purcell et al. 2012). They appear to modify their central seasonal peak, shifting their annual pattern appearance, commonly in summer (unimodal peak), to spring and summer (bimodal peak) (D'Ambrosio et al. 2016)

Recently, Marques et al. (2018) marked an essential assessment in the Mondego estuary, relating the interactions between climate and plankton. According to the authors, after 2008 a modification was noticed in planktonic communities due to significant changes in hydroclimate forcing. As stated above, the positive phase of the NAO modifies the hydroclimatic environment on the northeast coast of Europe, increasing the activity of NW Iberia anticyclone and generating visible alterations in the upwelling index (Santos et al. 2011). According to Marques et al. (2018) “the influence further affects river flow regimes, which tend to be lower (higher) when the NAO is in its positive (negative) phase”. In the same way, the primary production increases subsequently with the rising of nutrients income from deep-waters, boosting zooplankton growth with optimal conditions. Because of that, the attended changes in the estuary promoted a rearrangement of the zooplanktonic structure after 2007, conducted by a declining presence of freshwater taxa and consequently an opportunistic development of marine species. In fact, the observed taxa responsible for these significant changes in zooplankton community were primarily gelatinous groups. These considerations about the rising of gelatinous zooplankton abundance are in accordance with other results in Northeast Atlantic coast unveiling a tight connection with environmental changes (Lynam et al. 2011; Reygondeau et al. 2015). This pattern could bring adverse effects to the ichthyoplankton community (Cushing 1990), as the increase of gelatinous organisms may diminish the primary food for fish larvae.

Regarding Marques et al. (2018), it is possible to understand the climate-plankton network interactions between two different scenarios, prior and after 2008. Firstly, the path diagram before 2008 reveals a positive association among NAO, salinity, water temperature and upwelling. Additionally, the upwelling index leverages the estuarine temperature (negative relation) and salinity (positive relation). Secondly, after 2008 the novel configuration shows a notable influence increase by the NAO on upwelling index, temperature and salinity conditions. Finally, it is possible to ensure that the observed changes have a profound impact on predation pressure, since the influence of Medusa upon appendicularians is profoundly negative, as well as chaetognaths on cladocerans community (Marques et al. 2018).

Overall, the statements above highlight the straight connection within the climate-plankton network in Mondego estuary, what seems to be strictly linked to climate forcing variations and its non-stationary behaviour, promoting a cascading effect from climate to zooplanktonic communities.

3. SPECIES DESCRIPTION

This study will focus on two different holozooplanktonic gelatinous groups, Tunicata and Chaetognatha. The first mentioned group is more commonly called by tunicates, but they represent the subphylum Urochordata which include around 1600 species, divided into three classes – Ascidiacea, Thaliacea and Appendicularia (Larvacea in some classifications). Here we will follow the abundance of *Fritillaria borealis* and *Oikopleura dioica*, two appendicularians which are pelagic with relatively simple/short life cycles and exhibit a high resemblance appearance with larval stages of other tunicates considering that their adult tadpole-like body is formed by a trunk and a tail, containing the notochord cells (Deibel and Lowen 2011; Hickman et al. 2008). These organisms are possible to distinguish between them through different characteristics in endostyle, stomach wall, pharyngeal perforations, oikoplast epithelium, tail and body shape. *O. dioica* is the only appendicularian which the sexes are separate (Fenaux 1963). The appendicularians are filter-feeders and they have a unique way to feed through a delicate house (build by a sphere of secreted mucous) surrounding the body (Flood 2003). Each house has passages that filter the water and drive

the organic compounds like nanoplankton to the animal's mouth. Thus, when this house of mucous become clogged with particulate organic matter, the animal abandons their house and build a new one (Alldredge 1976). Also, that abandoned house has an important role as a vector of energy transfer (Gorsky 1998; Gorsky et al. 2005) since their drifting constitutes a food source for other zooplanktonic groups such as copepods (Alldredge 1976), as well as a particulate organic matter itself when sinking to the deep sea (Acuna et al. 1995; Acuna and Kiefer 2000). When the availability of food is high, the appendicularians can build dense populations likewise other tunicates. These organisms are highly distributed and are possible to found all over the world's ocean (normally in the photic zone) (R Fenaux 1998), coastal waters and estuaries (Costello and Stancyk 1983; Hoover et al. 2006; Lindsay and Williams 2010).

Regarding the second gelatinous zooplanktonic group mentioned above, the chaetognaths will be represented in this study by *Parasagitta friderici*. This group is commonly known by arrow-worms and by their elongated torpedo-like body shape (Hickman et al. 2008). Chaetognaths are worldwide distributed and they are, by far, one of the most abundant planktonic predators (Bone et al. 1991). Furthermore, most of all are planktonic and reach a size length of a few mm. Although, there are some genera living in benthic and deep-sea waters that reach a body length of 120 mm (Ball and Miller 2006). Regarding Ball and Miller (2006), the resemblance across the phylum is relatively constant but it could differ considerably in some little details. These organisms appear to react mostly to vibrations in their environment. Indeed, they are voracious predators and it allows them to hunt in complete darkness; that's why their predator behaviour it's higher during the night (Baier and Purcell 1997; Horridge and Boulton 1967). Apparently, they eat everything that fits in their mouths like appendicularians or fish larvae (ichthyoplankton), but their main preys are copepods (Baier and Purcell 1997; Horridge and Boulton 1967). As was stated by Horridge and Boulton (1967), to strike their preys chaetognaths retract their hoods that cover the spines, allowing them to form a net and surround the prey, bringing them to their mouths surrounded by teeth. These organisms lack a respiratory and excretory system, thus solely diffusion oxygen ensures their tissues dependence (Ball and Miller 2006; Hickman et al. 2008). Usually, their nictemeral routine encompasses to stay close to the surface during the night and swim to deeper waters during daytime (Hickman et al. 2008). When their

abundances increase, which is frequent, their ecological impact could be noteworthy concerning another zoo- and ichthyoplankton communities.

4. MATERIAL AND METHODS

4.1 Study site

The study was conducted in the Mondego Estuary (40°08'N, 8°50'W), located in the western Atlantic Coast of mainland Portugal. This is a mesotidal system, showing a Mediterranean temperate climate, where the NAO strongly dominates atmospheric variability (Trigo et al. 2004; Marques et al. 2018). This shallow system presents a hydrological basin area of 6670 km², yielding an average freshwater flow rate of 79 m³.s⁻¹ (Dolbeth et al. 2010).

The Mondego Estuary presents two arms (north and south) branched about 7 km from the sea, separated by Murraceira Island, showing different depths between them and converging again near the mouth of the estuary. The northern arm is deeper (4-8 m at high tide) than the southern one, and represents the main channel for navigation, linking directly to the Mondego River. In contrast, the southern arm is shallower (2-4 m at high tide), and the navigation traffic permutes due to tides influence or by freshwater flow inputs from a small affluent, regulated by a sluice, the Pranto River (Cardoso et al. 2004; Grilo et al. 2012). A whole description of this system is fully detailed in Marques et al. (2006).

4.2 Biological data

The zooplanktonic surveys were collected every month, at high tide from January 2003 to December 2012, at five downstream sampling stations of the Mondego Estuary (M – mouth; N1 and N2 – northern arm; S1 and S2 – southern arm) (**Figure 1**). Particularly, the stations near the mouth of Mondego estuary are the ones more exposed to the ocean-climate influence.

The collecting moment was done by subsurface horizontal tows with a plankton net (mesh size 335 µm; opening diameter: 0.5 meters; tow length: 3 minutes; tow speed: 2 knots), performing one replicate per haul in each station. Hydro-Bios flowmeter was used attached

to the entrance of the net, in order to estimate the average of filtered water volume ($39 \pm 22 \text{ m}^3$) (Marques et al. 2006). Also, water samples were collected for chlorophyll-*a* concentration ($\text{Chl. } a, \text{ mg.m}^{-3}$) to ascertain total phytoplankton biomass.

The samples were fixed with 5% buffered formaldehyde; switched and stored to 70% ethanol at the lab; samples were sub-sampled using a Folsom splitter and the species identified through a dissecting microscope. Specimens identification was performed, if possible, to the lower taxonomic level. Appendicularians, chaetognaths and their preys (estuarine and marine copepods) were counted and used in data analysis. Furthermore, the density was estimated and expressed as the number of organisms per one hundred cubic meters (ind.100m^{-3}).

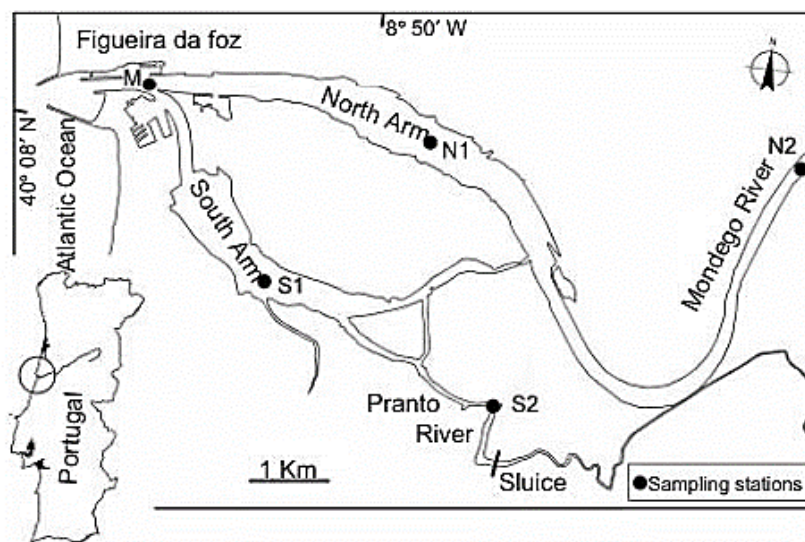


Figure 1: Location of collecting stations (M - mouth station; N1 and N2 - northern arm stations; S1 and S2 - southern arm stations) in Mondego Estuary.

4.3 Environmental data

The hydrological variables such as estuarine water temperature ($^{\circ}\text{C}$), salinity and dissolved oxygen (DO) (mg. L^{-1}) were registered using appropriate probes (WTW Cond 3310) at the same time as plankton samples were collected.

Since no meteorological station was present in the study area, all freshwater runoff (dam^3) and precipitation (mm) data were obtained from the Portuguese Water Institute (SNIRH, <https://snirh.apambiente.pt>) stations Soure 13/01G and Açude Ponte de Coimbra 12G/01A (near the city of Coimbra). According to the reports of the Portuguese Institute for Sea and Atmosphere (IPMA, www.ipma.pt/), three major drought events were considered along the study period: 2004-2005, 2007-2008 and 2012 (Garcia-Herrera et al. 2007; Trigo et al. 2013).

4.4 Statistical analysis

Species abundance was logarithmic transformed in order to approach a normal distribution and to minimize the dominant effect of exceptional catches (Legendre and Legendre 1998). Interannual variability of biological and environmental variables was represented by standardized anomalies (z-scores), computed as deviations from the mean of the time-series and divided by the respective standard deviation. The cumulative sum approach (CUSUM) of the deviations from the mean of reference were used concerning the detection of changing points in the species abundance and in the environmental parameter's interpretation. These variations on the slope's steepness and their signs, reflected the deviation of a certain period from the respective time-series mean value (Ibanez et al. 1993). Pearson's correlation analysis was performed to identify the relationship between environmental variables and species parameters. Linear regression was used to estimate the relationship between chaetognaths (predator) and copepods (prey).

5. RESULTS

5.1 Seasonal environmental variability

During the study period, the environmental conditions showed a wide range pattern presenting extreme climate events. Three drought periods were distinguished, namely over 2004-2005, 2007-2008 and 2012. In accordance with IPMA, the drought period of 2004-2005 was the most severe, affecting both environmental and socio-economic sectors (<https://ipma.pt>) (Garcia-Herrera et al. 2007; Trigo et al. 2013).

Throughout the study, the Mondego estuary exhibited a regular pattern of runoff ($138741 \pm 172156 \text{ dam}^3$) with low and high values, over the summer and winter seasons, respectively. Unexpectedly, both minimum (17018 dam^3) and maximum (729870 dam^3) values were reached in the winter of 2012 and 2003, respectively (**Figure 2A**). On the other hand, the salinity (21 ± 8) exhibited an opposing pattern, once it reached its minimum (4) in the autumn season of 2006, and its maximum (31) in the spring of 2005 (**Figure 2A**).

The precipitation factor ($55 \pm 37 \text{ mm}$) followed an expected pattern, reaching its minimum (0.2 mm) in the summer of 2009 and 2010, and its maximum (140 mm) in the autumn season of 2006 (**Figure 2B**). Estuarine water temperature ($17 \pm 3 \text{ }^\circ\text{C}$) presented a clear seasonal pattern too, reaching its high values in hot seasons and low values in the cold ones. The maximum water temperature value was reached in the summer of 2004 ($22 \text{ }^\circ\text{C}$), and the minimum ($12 \text{ }^\circ\text{C}$) in the winter of 2003 (**Figure 2B**).

Furthermore, the dissolved oxygen ($8.8 \pm 1.1 \text{ mg. L}^{-1}$), tended to decrease (minimum – 6.2 mg. L^{-1}) during the hot seasons and increase (maximum – 13.0 mg. L^{-1}) in the coldest ones. Despite this fact, the DO values tend to increase throughout the study period (**Figure 3**).

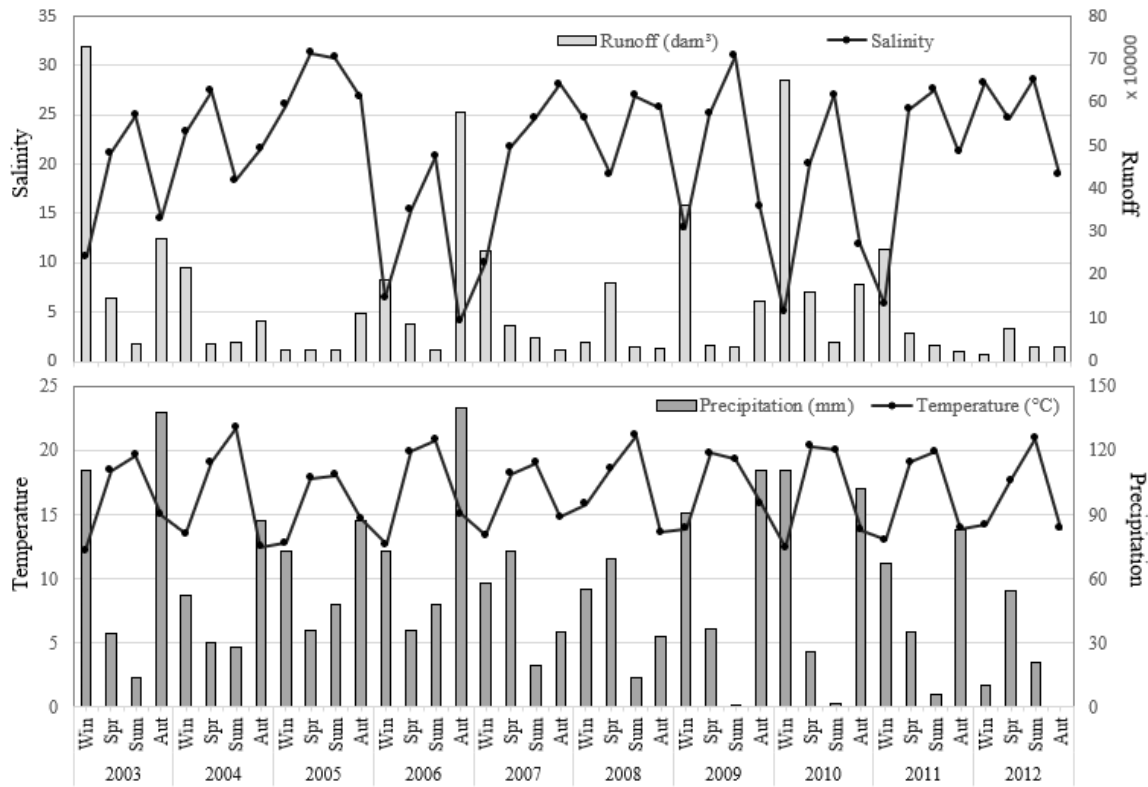


Figure 2: Seasonal mean values of runoff and estuarine salinity (A); precipitation and estuarine water temperature (B) during the study period in the Mondego estuary. Winter (Win) season was represented by January, February and March; Spring (Spr) season was represented by April, May and June; Summer (Sum) season was represented by July, August and September; and Autumn (Aut) season was represented by October, November and December months.

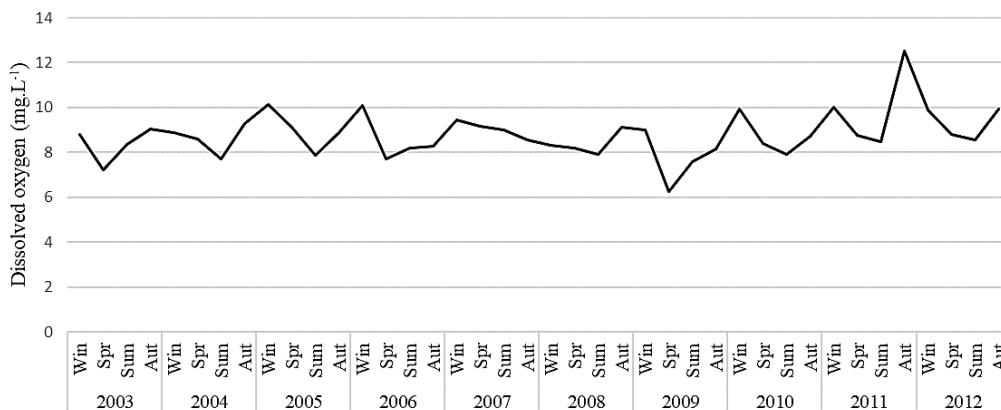


Figure 3: Seasonal variability of dissolved oxygen (mg. L⁻¹) during the study period in the Mondego estuary.

Concerning the interannual variability of the environmental factors, water temperature exhibited an increasing trend all over the study period (**Figure 4A**). In regard to salinity, their seasonal values showed three marked periods with positive slopes between 2005-2006, 2007-2008 and 2011-2012 (**Figure 4B**). The chlorophyll *a* exhibited an evident upward trend starting in the summer of 2006 up to the end of 2008 (**Figure 4C**). CUSUM analysis of DO exhibited a decrease until 2011 when it started to increase (**Figure 4D**), while the precipitation showed high variability with two main upward periods during the end of 2006 and 2009 (**Figure 4E**). In relation to runoff, it showed two increasing periods following the precipitation pattern, and three decreasing periods over the drought years of 2004-2005, 2007-2008 and 2011-2012 (**Figure 4F**).

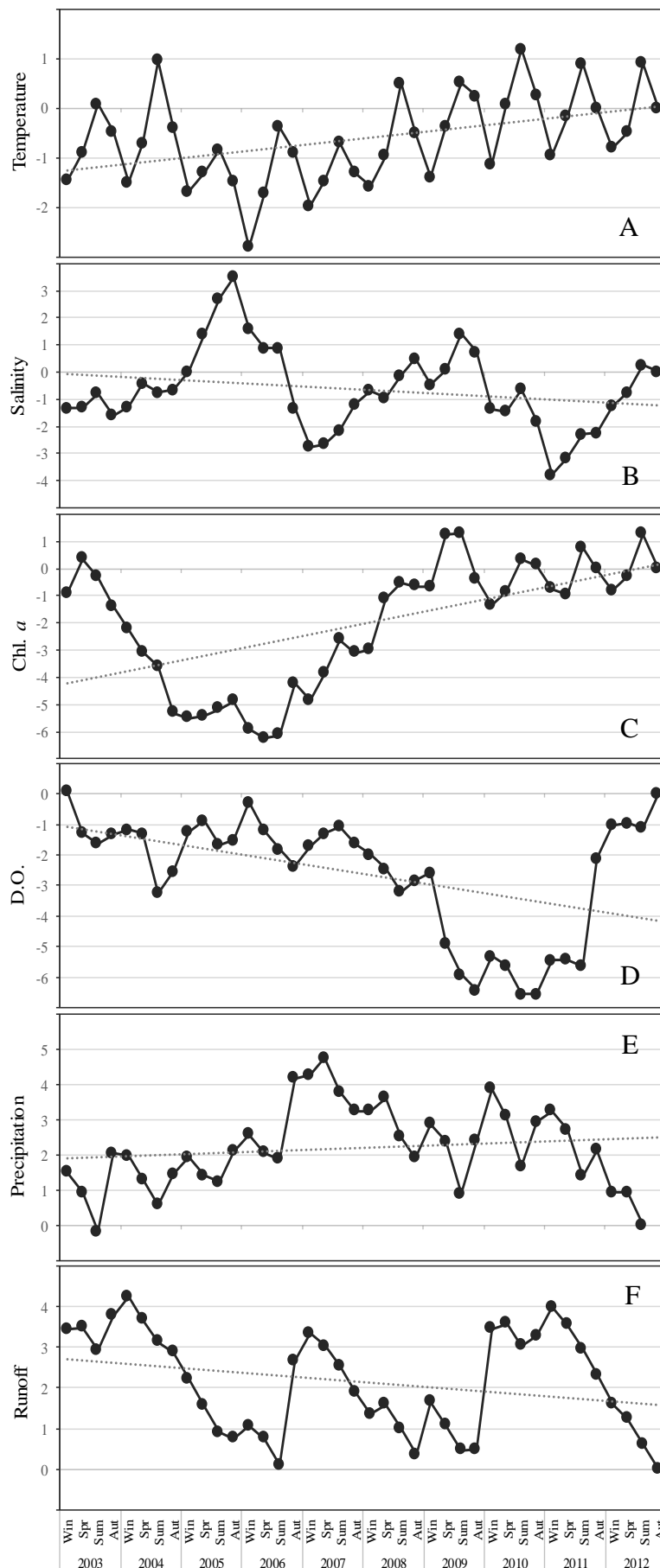


Figure 4: Cumulative sums standardised anomalies (z-scores) of long-term average: (A) water temperature, (B) salinity, (C) chlorophyll a (Chl. *a*), (D) dissolved oxygen (DO), (E) precipitation and (F) runoff; from 2003 to 2012 (winter - Win, spring - Spr, summer - Sum and autumn - Aut). The trend lines (dashed lines) were also added to understand the environmental factors tendency along the time series.

The analysis of the environmental variables' correlations (**Table 1**) revealed that temperature was the most correlated variable, exhibiting a strong significant positive correlation with Chl. *a* ($r = 0.564, p < 0.001$) and with salinity ($r = 0.491; p < 0.01$). Furthermore, temperature was negatively correlated with dissolved oxygen ($r = -0.658; p < 0.001$), precipitation ($r = -0.596; p < 0.001$) and runoff ($r = -0.487; p < 0.05$). Regarding salinity, this variable presented a significant negative correlation, with precipitation and runoff ($r = -0.669$ and $r = -0.771, p < 0.001$, respectively). Chl. *a* showed a negative significant correlation with DO ($r = -0.460; p < 0.01$). Lastly, precipitation exhibited a positive correlation with runoff ($r = 0.690; p < 0.001$).

Table 1: Pearson correlation between the environmental variables: temperature, salinity, chlorophyll *a* (Chl. *a*), dissolved oxygen (DO), precipitation and runoff. The values presented in the table follow the next order: correlation coefficient – r ; p -value and number of samples. Asterisk means the significant p -value level (e.g. $p < 0.001$ stands in bold; (***) stands for $p < 0.01$ and (**) stands for $p < 0.05$).

	Temperature	Salinity	Chl. <i>a</i>	D.O.	Precipitation	Runoff
	0,4910	1				
Salinity	***0,0013					
	40					
	0,5640	0,2700	1			
Chl. <i>a</i>	<0,001	0,0925				
	40	40				
	-0,6580	-0,2260	-0,4600	1		
D.O.	<0,001	0,1610	***0,00281			
	40	40	40			
	-0,5960	-0,6690	-0,2420	0,2860	1	
Precipitation	<0,001	<0,001	0,1330	0,0740		
	40	40	40	40		
	-0,4870	-0,7710	-0,1270	0,1240	0,6900	1
Runoff	***0,00144	<0,001	0,4350	0,4450	<0,001	
	40	40	40	40	40	

5.2 Biological data

5.2.1 Species abundance throughout the study period

During the study period, species abundance displayed a conspicuous variability at seasonal and interannual scales, reaching their higher densities during the hot seasons (e.g. spring and summer). For instance, the Tunicata *Oikopleura dioica* showed up higher densities among the three species and *Fritillaria borealis* was the species with lower recorded density (**Table 2** and **Figure 5**). During the study, the mean density of *Fritillaria borealis* reached 20 ± 214 ind.100m⁻³ and their maximum (4647 ind.100m⁻³) was obtained in the summer of 2009. The Chaetognatha, *Parasagitta friderici* mean density was 276 ± 941 ind.100m⁻³ and reached their maximum (12630 ind.100m⁻³) during the spring season in the last recorded year. *Oikopleura dioica* reached a mean density of 305 ± 883 ind.100m⁻³ and a maximum of 9320 ind.100m⁻³ in the summer of 2009.

Regarding the year of 2004 the summer samples (represented by the months of July and August) were not sampled, only September was considered.

Table 2: Decadal mean (\pm standard deviation) and maximum abundances (ind.100m⁻³) during the 10-year time series.

Taxa	(2003 – 2012)		
	Mean	SD	Max.
<i>Fritillaria borealis</i>	20	214	4647
<i>Oikopleura dioica</i>	305	883	9320
<i>Parasagitta friderici</i>	276	941	12630

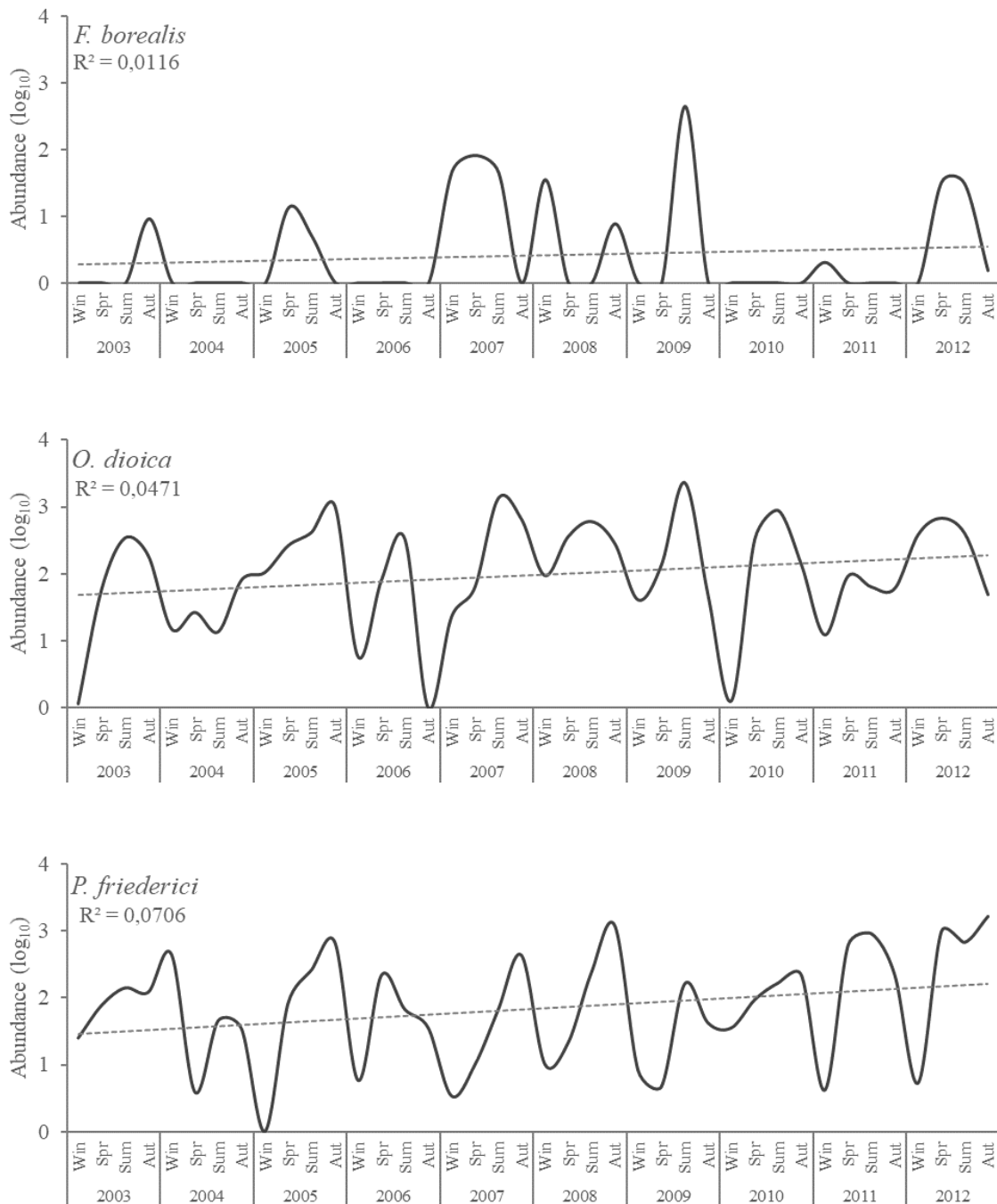


Figure 5: Interannual and seasonal abundance (ind.100m⁻³) logarithmic transformed, of the three studied species. Dashed lines (and R²) represents the decadal trend of each species.

Concerning species distribution, their abundances prevailed in the middle areas' stations, near to the mouth of the Mondego estuary (e.g. stations M, N1 and S1). This fact can possibly be explained by the marine nature of the species and the higher levels of salinity since the maximums were all recorded in the M and S1 station (**Figure 6**). In addition, no significant statistical differences were found ($p > 0.05$) for *Fritillaria borealis* densities, and their major mean peak abundance ($393.48 \pm 1339.54 \text{ ind.}100\text{m}^{-3}$) was recorded in the mouth station during the year of 2009 (**Figure 6A**). *O. dioica* and *P. friderici* showed significant differences ($p < 0.01$) only in the S1 site when compared to the S2 (**Figure 6B** and **C**). *O. dioica* showed up a few abundance peaks during the study period, but none of them was statistically different over the years. However, this appendicularians exhibited some conspicuous values in terms of annual mean abundance at the mouth, middle and upper estuary (N2). Looking at Figure 6, at the mouth station, in 2009 and 2010 they register a mean density of $584 \pm 1213 \text{ ind.}100\text{m}^{-3}$ and $657 \pm 1888 \text{ ind.}100\text{m}^{-3}$, respectively. At the N1 station, the *Oikopleura dioica* reached up to $765 \pm 1812 \text{ ind.}100\text{m}^{-3}$, $464 \pm 785 \text{ ind.}100\text{m}^{-3}$, $575 \pm 892 \text{ ind.}100\text{m}^{-3}$ and $617 \pm 1390 \text{ ind.}100\text{m}^{-3}$, in the years of 2005, 2007, 2008 and 2009, respectively. Regarding S1 station, two main peaks were registered in 2007 with $1266 \pm 2701 \text{ ind.}100\text{m}^{-3}$, and 2009 revealing a mean density of $1475 \pm 2725 \text{ ind.}100\text{m}^{-3}$.

Parasagitta friderici was observed every year, but the most recent years of the study period were the ones with the highest densities. Their mean densities reached some interesting values in the mouth and middle estuary sites (**Figure 6C**). In the mouth site, they reveal a mean density peak of $2010 \pm 4163 \text{ ind.}100\text{m}^{-3}$ in 2012 and marked their maximum density ($12630 \text{ ind.}100\text{m}^{-3}$) at this site during this year. In the N1 site, two mean abundance peaks were registered of $874 \pm 1470 \text{ ind.}100\text{m}^{-3}$ and $930 \pm 2209 \text{ ind.}100\text{m}^{-3}$, in 2011 and 2012, respectively. In the middle estuary, at the S1 station, the *Parasagitta friderici* reached a mean abundance peak ($580 \pm 1400 \text{ ind.}100\text{m}^{-3}$) in 2008. Yet, in the years of 2011 and 2012, they doubled that value with $1151 \pm 1425 \text{ ind.}100\text{m}^{-3}$ and $1139 \pm 1747 \text{ ind.}100\text{m}^{-3}$ mean densities, respectively.

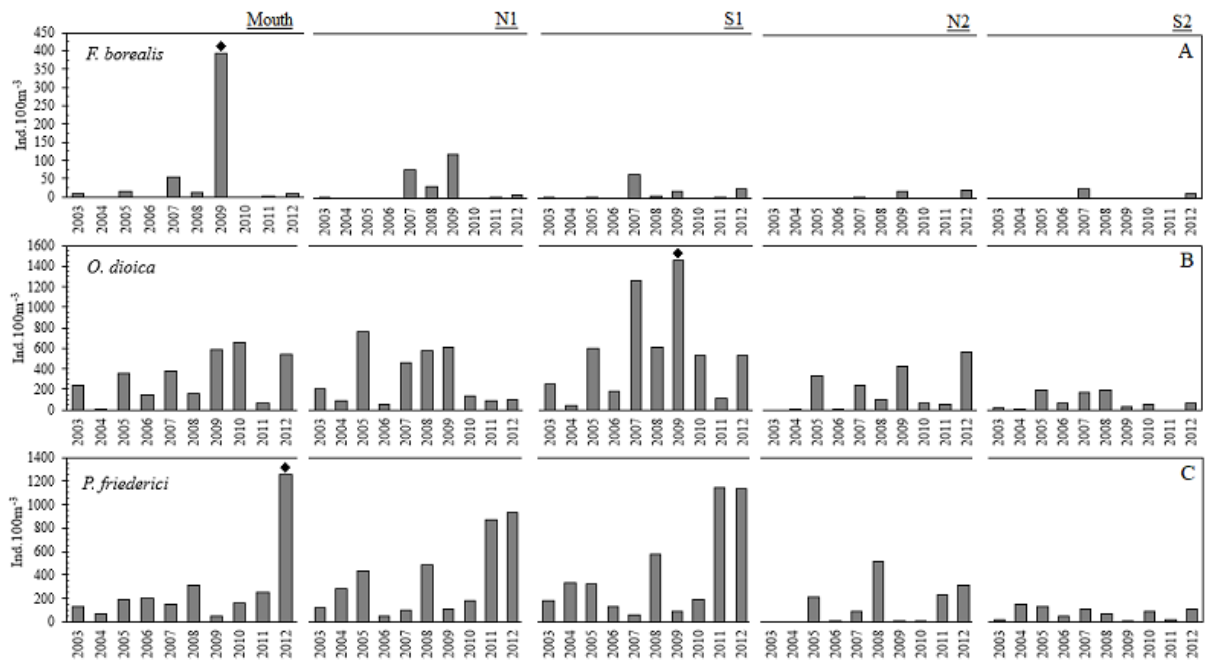


Figure 6: Annual mean density (ind.100m⁻³) of each species at the five sites (mouth area - M; middle area - N1 and S1; upper/inner area - N2 and S2) during the study period. A) *Fritillaria borealis*; B) *Oikopleura dioica*; C) *Parasagitta friderici*. Black diamonds (◆) represents the year, and site, where the maximums of each species were reached.

5.2.2 Interannual distribution and drought events

In the heat map (**Figure 7**) is represented the spatial distribution, showing an expressive difference in their densities after 2007-2008 drought years. *F. borealis*, which presented the lowest densities, showed an increasing abundance during the following year after 2008 drought events. In Figure 7, it is possible to observe *O. dioica* and *P. friderici* occurrence. They showed high numbers in the estuary entrance and middle, and their presence was clearly high after 2008. Through a simple analysis, comparing means, standard deviations and maximums in different periods before (2003-2008) and after (2009-2012) drought events, the outcome was predictable as we expected (**Table 3**). *F. borealis*, between the two periods, increase the mean abundance, and their standard deviation increased by approximately 450%. Secondly, the mean abundance of *O. dioica* increased more than 120%. Thirdly, the chaetognath *P. friderici*, more than doubled their mean and standard deviation, between these two periods separated by the drought of 2007 and 2008.

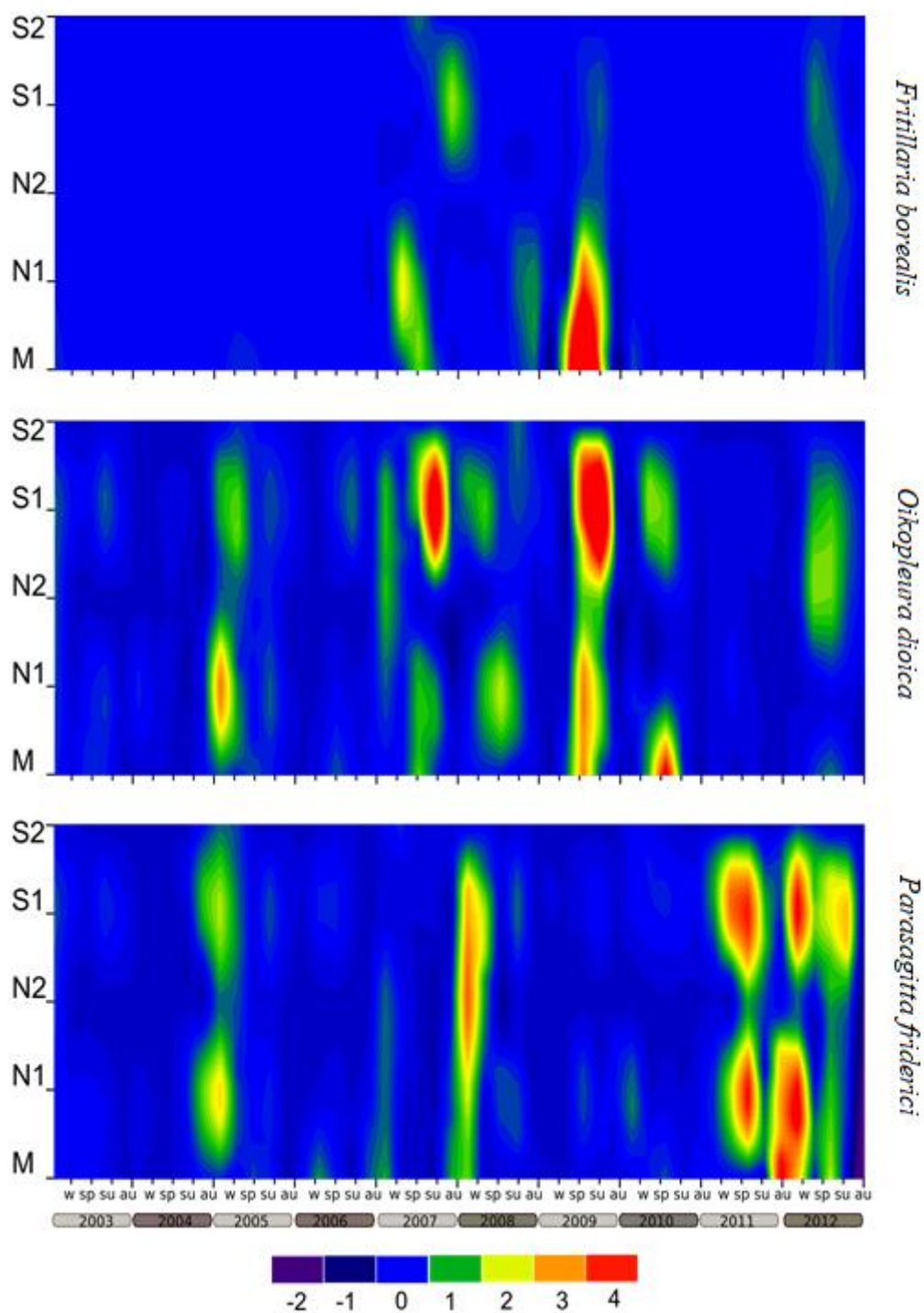


Figure 7: Density anomalies heat map – Total density anomalies of each species distribution along the study period, subdivided by seasons (w - winter; sp - spring; su – summer; au - autumn). It is possible to recognize an abundance increase, after 2007-2008 years, of all species.

Table 3: Mean (\pm standard deviation) and maximum abundances (ind.100m⁻³) comparisons between two periods before and after the 2008 drought event.

Taxa	(2003 – 2012)		(2003-2008)			(2009-2012)		
	Mean	SD	Mean	SD	Max.	Mean	SD	Max.
<i>F. borealis</i>	20	214	11	71	842	32	315	4647
<i>O. dioica</i>	305	883	274	759	8971	347	1025	9320
<i>P. friderici</i>	276	941	187	565	4974	393	1272	12630

5.2.3 Temporal variability of Tunicata and Chaetognaths in Mondego estuary

Throughout the study period, it was possible to notice an increasing abundance of *O. dioica* and *P. friderici* (**Figure 8A** and **B**) as stated above. As regards the CUSUM, the species appear to be rising, through the inclination and persistence of their slopes. On the other hand, *F. borealis* exhibited two negative slopes during the first four years and afterwards during the years 2010-2011 (**Figure 8C**). Although, their maximum abundance in 2009 was very evident.

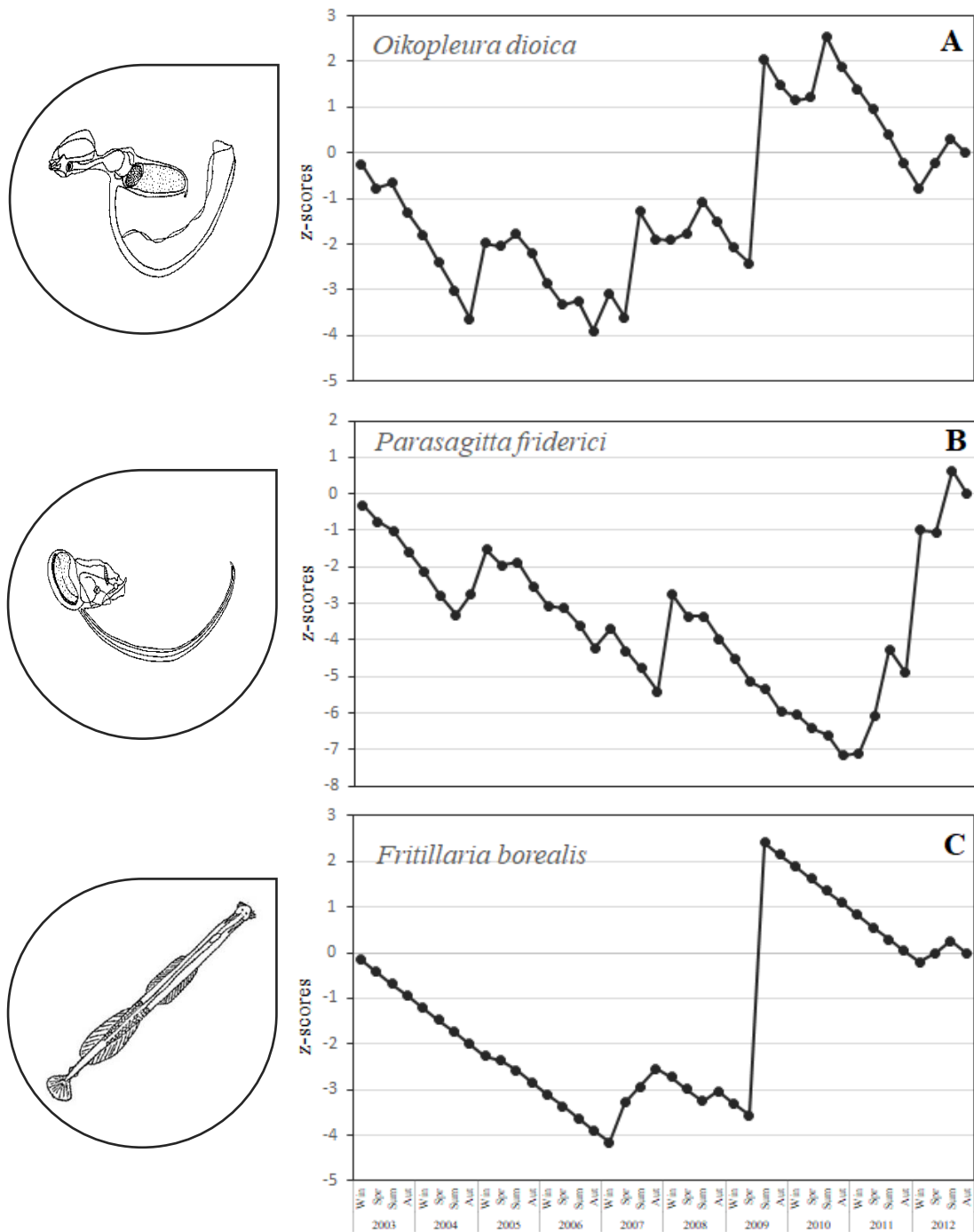


Figure 8: CUSUM - Cumulative sums of standardised anomalies (z-scores) of long-term abundance average - (A) *Oikopleura dioica*; (B) *Parasagitta friderici*; (C) *Fritillaria borealis*. Species illustrations adapted from Newell and Newell (1963) in Ré et al. (2005).

O. dioica and *P. friderici* showed a lot of positive years where their densities were well above the long-term average, and negative years with low densities below the long-term average (**Figure 9A and B**). Also, *P. friderici* showed a slight tendency to increase their density anomalies.

Regardless of the *F. borealis* minor densities, they exhibited an increase (above the long-term average) in their densities only in the years 2007 and 2009 (**Figure 9C**).

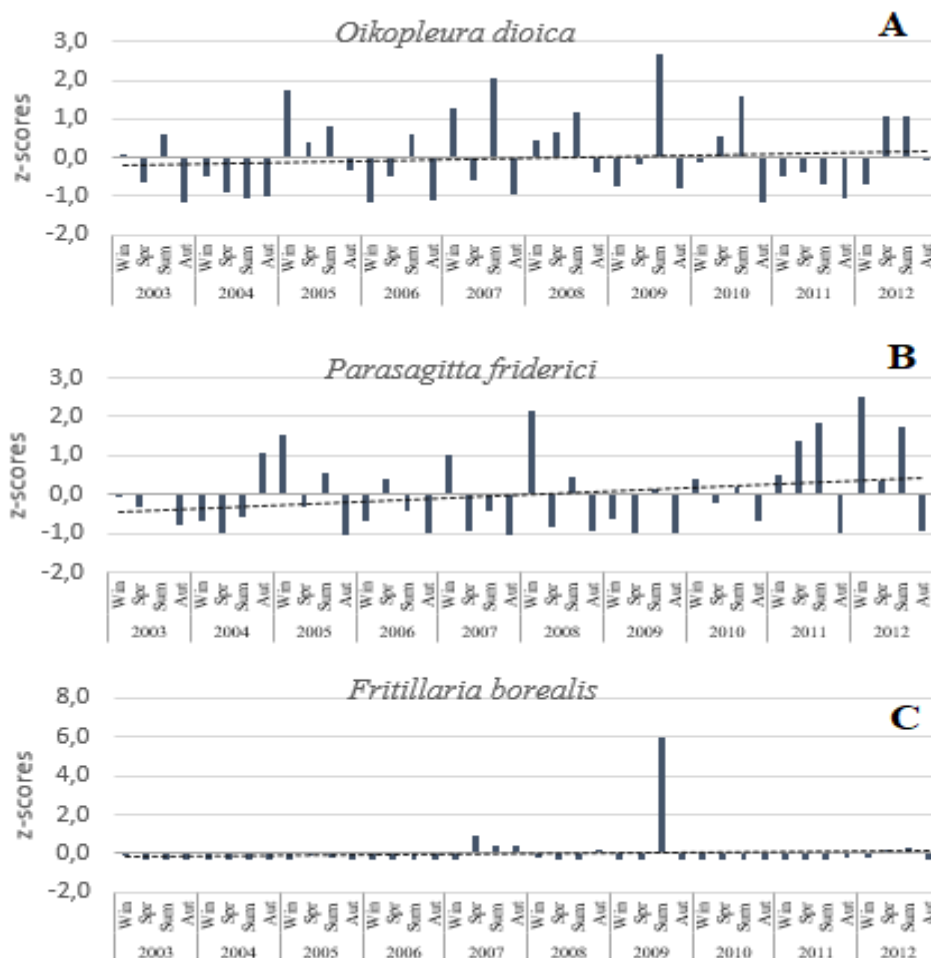


Figure 9: Standardised anomalies of long-term densities average (z-scores); (a) *Oikopleura dioica*; (b) *Parasagitta friderici*; (c) *Fritillaria borealis*. Trend-lines were also included (dashed lines).

5.2.4 The predator-prey relationship and the environmental variability impact

Our interest was directly connected to the relationship between *P. friderici* and their preys, which were also included the appendicularians, due to the chaetognaths carnivorous behaviour (Baier and Purcell 1997; Hickman et al. 2008). Therefore, the Pearson correlation results revealed that *P. friderici* was negatively correlated with *F. borealis* and *O. dioica* ($r = -0.252$ and $r = -0.364$, respectively). On the other hand, the Chaetognatha also showed a positive correlation with estuarine and marine copepods ($r = 0.380$ and $r = 0.374$, respectively) (**Table 4**).

Table 4: Pearson correlation, using standardized CUSUM data, between predators (*P. friderici*) and their preys (appendicularians, estuarine and marine copepods). The outcomes are presented with three different values – correlation coefficient - r , p -value and number of samples. Statistically significant values are in bold.

	<i>F. borealis</i>	<i>O. dioica</i>	Estuarine Copepoda	Marine Copepoda
	-0,252	-0,364	0,380	0,374
<i>P. friderici</i>	< 0,001	< 0,001	< 0,001	< 0,001
	199	199	199	199

Statistically significant (positive) relationship was found between *P. friderici*, *O. dioica* ($p < 0.05$) and marine copepods ($p < 0.001$) (**Table 5**). Concerning the same analysis, *Fritillaria borealis* and estuarine copepods were not significantly related to chaetognaths.

Table 5: Multiple linear regression – $N = 200$; $R = 0.614$; Std. Error of estimate = 0.846. Asterisk means the significant p -value level (e.g. $p < 0.001$ stands in bold; (***) stands for $p < 0.01$ and (**) stands for $p < 0.05$). Dependent variable – *Parasagitta friderici*.

	Coefficient	Std. Error	t	P	VIF
Constant	-0,552	0,262	-2,111	0,036	
<i>Fritillaria borealis</i>	-0,080	0,101	-0,793	0,429	1,112
<i>Oikopleura dioica</i>	0,233	0,075	3,121	** 0,002	1,839
Estuarine Copepod	0,044	0,041	1,073	0,285	1,027
Marine Copepod	0,483	0,081	5,986	< 0,001	1,749

Concerning the relationship between the biological and environmental variables, the Pearson correlation revealed that DO and Chl. *a*, were positively correlated ($p < 0.001$) with all species (**Table 6**). *P. friderici* exhibited a positive correlation with DO and negatively correlated with Chl. *a*. On the other hand, both appendicularians exhibited the opposite pattern mentioned above. Water temperature presented positive significant correlation with *F. borealis* and *O. dioica* ($p < 0.001$, $r = 0.590$ and $r = 0.566$, respectively), while with *P. friderici* presented a negative correlation ($p = 0.05$, $r = -0.159$). Regarding salinity, only *P. friderici* were statistically significant ($p < 0.01$) in this analysis and displayed a negative correlation with this environmental variable (**Table 6**).

Table 6: Pearson correlation of mean annual anomalies cumulative sum (CUSUM), confronting biological data (*F. borealis*; *O. dioica* and *P. friderici*), environmental variables (water temperature, salinity and DO) and phytoplankton (estimated by Chl. *a*). The outcomes are presented with three different values – correlation coefficient - *r*, *p*-value and number of samples. Statistically significant values are in bold. Asterisk means the significant *p*-value level (e.g. $p < 0.001$ stands in bold; (***) stands for $p < 0.01$ and (**) stands for $p < 0.05$).

	Water temperature	Salinity	DO	Chl. <i>a</i>
	0,590	-0,115	-0,304	0,682
<i>Fritillaria borealis</i>	< 0,001	0,107	< 0,001	< 0,001
	199	199	199	199
	0,566	0,004	-0,380	0,812
<i>Oikopleura dioica</i>	< 0,001	0,953	< 0,001	< 0,001
	199	199	199	199
	-0,159	-0,214	0,756	-0,276
<i>Parasagitta friderici</i>	**0,025	**0,002	< 0,001	< 0,001
	199	199	199	199

6. DISCUSSION

In recent years there has been considerable evidence showing the relationship between the climate variability and the abundance of gelatinous carnivore zooplankton (Calbet 2001; D'Ambrosio et al. 2016; Molinero et al. 2008; Primo et al. 2012; Kang and Ohman 2014; Kusum et al. 2014). Our results are consistent with these evidence since both *Oikopleura dioica* and *Parasagitta friderici*, fully matched some previous results with other gelatinous species (Calbet 2001; Kusum et al. 2014). It is worth noting that all studied species were correlated with temperature (**Table 6**) and *Parasagitta friderici* (Chaetognatha) displayed a negative correlation with this variable, perhaps driven by their affinity with cold-waters as suggested by Molinero et al. (2008). Likewise, a positive significant correlation between *P. friderici* and dissolved oxygen was found. This fits with Kusum et al. (2014) outcomes in the Indian Ocean and also with Calbet (2001) since chaetognaths and appendicularians were equally correlated with water temperature, in NW Mediterranean sea. Regarding Kusum et al. (2014) findings, *Sagitta spp.* revealed negative and positive association with temperature and DO in the upper layer, respectively. Thereby, these results further alert for global warming, once the effect is slower in deeper layers of the water column than in the water surface (Kusum et al. 2014). The effects described above could influence the seasonal abundance peaks, changing their patterns and favouring the mismatch between different trophic levels (Edwards and Richardson 2004) and subsequent poor fish recruitment (Bucklin et al. 2010) due to chaetognaths voracious predation behaviour (Baier and Purcell 1997) upon ichthyoplankton and their preys (Baier and Purcell 1997). Chaetognaths were also negative correlated seasonally with appendicularians, presumably caused by that predation pressure and/or the opposite affinity with water temperature.

In terms of the three species abundance, *O. dioica* revealed to be the most abundant, which is a good evidence considering their important role as a vector of energy transfer generated by the sinking of their gelatinous houses (Acuna et al. 1995; Acuna and Kiefer 2000). *O. dioica* and *P. friderici* displayed similar seasonal peaks and clearly their densities increased after the drought years 2007-2008 as well as their variability diminished after that period. The summer season was undoubtedly the season with higher abundances, followed by winter, spring and autumn, respectively (**Table 7**). Although, *P. friderici* presented a summer-autumn distribution which nearly coincided with the abundance peaks of *O. dioica*,

as was stated as well by Calbet (2001). In the same way, **Table 7** shows some of the resemblance years (2005, 2008, 2009 and 2012) between *O. dioica* and *P. friderici* in the abundance peaks, during the study period. In addition, when confronting both species, *P. friderici* was more abundant during the winter season, and *O. dioica* reveals major densities during summer. Furthermore, the appendicularians were positively correlated with Chl. *a*, which were predictable, due to their filter-feeding behaviour (Aravena and Palma 2002; Flood 2003), while in the NW Mediterranean sea Calbet (2001) recorded negative correlations between appendicularians and Chl. *a*.

Statistically significant correlations were verified between chaetognaths and marine copepods, likewise the results of Kang and Ohman (2014). In this way, we expected a strong predation-prey relationship (concerning the results obtained in the multiple linear regression) between chaetognaths, appendicularians and marine copepods, most of all, because *P. friderici* is recognized as a primary carnivore. Still, Molinero et al. (2008) suggest that in the NW Mediterranean sea, chaetognaths population variability and/or growth could be also related to the predation pressure by jellyfish. The overlapping of jellyfish, copepods and appendicularians abundance peaks (due to warmer waters) may decrease directly and/or indirectly the chaetognaths abundance. This pressure could be caused by the lack of food availability or being predated by jellyfish (Molinero et al. 2008).

Regarding Mondego estuary previous studies showed a connection between drought events and zooplankton species abundance (Primo et al. 2015), our results also reveal differences between the abundance means and maximums before and after the drought years (e.g. *O. dioica* and *P. friderici*, in the years 2005 and 2007 - **Table 7**). The same conclusions have been verified by D'Ambrosio et al. (2016) concerning other gelatinous species *Muggiaea atlantica* (Siphonophora) in this estuary. Given that, there have been many results showing high correlations between gelatinous zooplankton and water temperature in Mondego estuary (Primo et al. 2012, 2015; D'Ambrosio et al. 2016). These works revealed that gelatinous zooplankton changed their annual main peak, from unimodal to a bimodal pattern, after the last severe drought event (D'Ambrosio et al. 2016). As was stated above, our findings presented a similar pattern since both species *O. dioica* and *P. friderici* revealed unimodal abundance peaks in some years (e.g. 2003, 2006 and 2009) and bimodal years in others, such as 2005, 2008, 2010, 2011 and 2012. Sometimes their presence in the Mondego estuary was also evident along three seasons in a row. Hence, aside from jellyfish abundance

Table 7: Interannual and seasonal mean density (ind.100m⁻³) of the study species, in the Mondego estuary. The annual total mean was calculated considering the three species together. ⁽¹⁾ *F. borealis* annual mean values were not considered.

		2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Seasonal Mean	Seasonal St. Dev.
Winter	<i>P. friederici</i>	120,87	30,11	685,26	34,72	435,38	1212,26	41,09	219,03	254,81	1678,80	471,23	682,22
	<i>O. dioica</i>	185,31	71,50	1030,63	0,00	650,42	281,31	46,40	135,42	76,99	51,79	252,98	432,39
	<i>F. borealis</i>	8,17	0,00	0,00	0,00	0,00	6,72	0,00	0,00	0,00	0,53	1,54	6,58
Spring	<i>O. dioica</i>	56,95	24,69	261,19	79,06	60,99	369,59	133,02	324,20	91,09	528,44	192,92	319,51
	<i>P. friederici</i>	76,35	2,81	77,84	217,94	8,87	17,93	3,67	93,11	615,15	212,60	132,63	327,06
	<i>F. borealis</i>	0,00	0,00	11,78	0,00	81,17	0,00	0,00	0,00	0,00	33,25	12,62	44,30
Summer	<i>O. dioica</i>	325,81	12,56	413,05	327,60	1320,35	598,40	2281,48	878,55	48,41	531,02	673,72	1032,43
	<i>P. friederici</i>	131,10	44,70	262,50	66,32	65,28	240,94	161,79	166,63	905,03	856,71	290,10	442,53
	<i>F. borealis</i>	0,00	0,00	3,90	0,00	44,29	0,00	445,45	0,00	0,00	38,58	53,22	231,99
Autumn	<i>P. friederici</i>	22,22	446,51	0,00	4,81	2,34	9,20	7,16	32,31	3,14	7,59	53,53	176,38
	<i>O. dioica</i>	0,12	13,95	103,31	4,73	24,74	92,58	39,56	0,25	11,18	153,20	44,36	81,76
	<i>F. borealis</i>	0,00	0,00	0,00	0,00	43,04	34,44	0,00	0,00	1,03	0,00	7,85	35,35
Annual Mean	<i>O. dioica</i>	142,05	30,68	452,04	102,85	514,13	335,47	625,12	334,61	56,92	316,11		
	<i>P. friederici</i>	87,63	131,03	256,40	80,94	127,97	370,08	53,43	127,77	444,53	688,93		
	<i>F. borealis</i>	2,04	0,00	3,92	0,00	42,12	10,29	111,36	0,00	0,26	18,09		
Total		77,24	53,90	237,46	61,26	228,07	238,61	263,30	154,13	167,24	341,04		
Total¹		114,84	80,85	354,22	91,90	321,05	352,78	339,27	231,19	250,73	502,52		

results that Primo et al. (2012) had found, our upshot showed as well that the year of 2004, was the year with lowest densities recorded. Yet 2004 was the year without recorded samples, so we consider the year 2006 as well, which was the second year with the lowest annual total mean densities registered. Individually, *O. dioica* exhibited lower densities throughout the year of 2004 and 2011; *P. friederici* showed lower abundance during the year of 2009. Possibly, that decrease in densities could be related to the decline of chlorophyll *a* and DO, in 2004 and 2009, respectively.

It would also be interesting for this study if the length of the studied organisms were measured as well as their growth rate and maturation stage. Thus, we would be able to understand the relationship of gelatinous zooplanktonic population growth under the effect of regional climate variability (Broms and Tiselius 2003). Also, understanding the predation pressure enforced by jellyfish upon chaetognaths, would be interesting, revealing with precision the factors (environmental, preys' abundance or predation) that lead to their interannual variability.

7. CONCLUSION

As was reported above, our main goal was to understand the relationship between the environmental factors and the two aforementioned gelatinous zooplanktonic groups, in the Mondego estuary. This estuarine system revealed changes in the biological communities and in the hydrological features throughout the study period (e.g. intensified salinization as well as the rising water temperatures). Thus changes were led by the increasing of oceanic waters in the Mondego estuary, favouring the abundance of marine species and subsequently gelatinous zooplankton (Primo et al. 2012; D'Ambrosio et al. 2016). Concerning to water-temperature, preceding studies related their variability with the prevailing signal of NAO (Molinero et al. 2008; Lynam et al. 2011; Marques et al. 2018). According to Marques et al. (2018), the NAO is significantly related to the temperature in the estuary and consequently with their communities. That was the major environmental factor, likewise the dissolved oxygen variability, leading to detectable changes in the gelatinous zooplankton. A crucial issue to detect drifts and tendencies in gelatinous zooplankton (e.g. jellyfish), according to Condon et al. (2012), is the absence of a well-defined guideline, mainly owned by the insufficiency of gelatinous zooplankton long-term data. Overall, this study contributes to that guideline, stating important information about these non-talked groups and the way that environmental factors affect their life-history in the Mondego estuary. Understand their resilience to extreme weather events it is of major importance to elucidate how it will shape their success in extreme environmental scenarios.

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