Universidade de Aveiro Departamento de Biologia
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Leonardo Valente dos Santos

Spatio-Temporal Dynamics of The Zooplankton Community of The Mondego Estuary

Dinâmica Espacio-Temporal da Comunidade de Zooplâncton no Estuário Do Mondego

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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 da Doutora Ana Marta dos Santos Mendes Gonçalves, Investigadora em Pós-Doutoramento, MARE, Departamento de Ciências da Vida, Universidade de Coimbra e Departamento de Biologia da Universidade de Aveiro, Doutor João Carlos de Sousa Marques, Professor Catedrático, MARE, Departamento de Ciências da Vida, Universidade de Coimbra, Doutor Fernando José Mendes Gonçalves, Professor Associado com Agregação, Departamento de Biologia da Universidade de Aveiro

Dedico este trabalho a minha família.

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Prof. Doutor Ulisses Manuel de Miranda Azeiteiro

Professor associado c/agregação, Departamento de Biologia, Universidade de Aveiro

Doutor João Miguel Magalhães Neto

Investigador Cientista convidado, MARE, Departamento das Ciências da Vida, Universidade de Coimbra

Doutora Ana Marta dos Santos Mendes Gonçalves

Investigadora em Pós- Doutoramento, MARE, Departamento das Ciências da Vida, Universidade de Coimbra / Departamento de Biologia & CESAM, Universidade de Aveiro

agradecimentos

Agradeço aos meus orientadores por me darem esta oportunidade Doutora Ana Marta dos Santos Mendes Gonçalves, Doutor João Carlos de Sousa Marques do IMAR-Instituto do Mar, c/o Departamento das Ciências da Vida, Universidade de Coimbra, Doutor Fernando José Mendes Gonçalves do Departamento de Biologia da Universidade de Aveiro. Com grande apreço agradeço a tudo a equipe do MARE que me recebeu, realizou todas as saídas de campo e disponibilizou todo equipamento necessário para a realização deste trabalho, em especial Doutor João Carlos de Sousa Marques, Doutor João Miguel Magalhães Neto, Doutora Ana Carla Garcia, Doutora Helena Veríssimo de Carvalho, Doutor Tiago Gonçalo Martins Verdelhos, Pablo Baria, Micael Neves.

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palavras-chave Zooplâncton, estuário, distribuição vertical, eventos climáticos, inundações, seca.

resumo

Os estuários são dos sistemas aquáticos mais valiosos e produtivos em termos de bens e serviços para o bem-estar humano. Nas últimas décadas, evidenciou-se a importância do plâncton (e principalmente do zooplâncton) na dinâmica das cadeias tróficas aquáticas. O zooplâncton desempenha um papel ecológico fundamental em termos de transferência de fluxos de biomassa e energia entre produtores primários e níveis tróficos superiores, contribuindo para a importância deste grupo em estudos ecológicos. Além disso, escalas espaço-temporais da estrutura e composição das comunidades de zooplâncton são um foco importante em estudos ecológicos. Assim, o objetivo principal deste trabalho foi estudar a dinâmica espaço-temporal e a distribuição vertical da comunidade de zooplâncton no estuário do Mondego (Portugal), com base em campanhas mensais realizadas de maio de 2012 a março de 2014, no fundo à superfície, com uma rede de 335 µm de poro, em preia-mar. Determinou-se ainda a influência de variáveis ambientais (por exemplo, temperatura, salinidade, concentração de nutrientes) na distribuição da comunidade de zooplâncton. A ocorrência de eventos climáticos extremos (condições de seca e inundação) durante o período de estudo também permitiu determinar efeitos das condições climáticas extremas na ecologia do zooplâncton. Os resultados mostraram variações na distribuição e nas abundâncias de vários grupos de zooplâncton relacionados com condições de seca e inundações registadas durante o período de estudo. A principal variação nos parâmetros ambientais foi nos valores de salinidade e temperatura, levando ao predomínio de espécies marinhas em períodos de seca. Foi possível também identificar que estas variações ocorrem tanto nas comunidades de fundo como de superfície, no entanto, não se registam variações tão drásticas na composição da comunidade de zooplâncton no fundo.

keywords

Zooplankton, estuary, vertical distribution, climate events, flood, drought.

abstract

Estuaries are among the most valuable and productive aquatic systems in terms of their services to human welfare. In the last decades it has been highlighted the importance of plankton (and mainly zooplankton) in the dynamic of aquatic food webs. Zooplankton plays a pivotal ecological role in terms of biomass and energy fluxes transference between primary producers and higher trophic levels, being raised the importance of this group in ecological studies. Furthermore, spatio-temporal scales of zooplankton communities' structure and composition are an important focus in ecological research. Thus, the main aim of this work was to study the spatio-temporal dynamics and vertical distribution of zooplankton community in the Mondego estuary (Portugal), based on monthly field surveys conducted from May 2012 to March 2014, at the bottom and surface, with a mesh net size of 335 μm, in high tide. In addition, the influence of environmental variables (e.g. temperature, salinity, nutrients concentration) in the distribution of zooplankton communities was determined. The occurrence of a extreme climate events (drought and flood conditions) during the study period also allowed examining the effects of the extreme weather conditions on zooplankton ecology. The results showed variations in the distribution and abundance of various zooplankton groups related to drought and flood conditions during the studied period. The main variation in environmental parameters was register to the salinity and temperature levels, leading to the predominance of marine species during drought events. It was also possible to identify that these variations occur in the bottom and surface communities, however, at the bottom was not registered so drastic changes compared to the zooplankton composition from the surface.

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1. General Introduction

There is extensive information available about zooplankton communities (Lam-Hoai et al., 2006; Edwards and Richardson, 2004; Kirby et al., 2007), some of them associated to the effects of eutrophication process (Anneville et al., 2007; Hsieh et al., 2011), extreme climatic events, such as severe droughts and floods (Gonçalves et al., 2012; Isari et al., 2007; Hampton et al., 2008; Richmond et al., 2007), and the impact of fisheries (Reid et al., 2000). Although, information relating the impacts of climate variations in the zooplankton community at the bottom and at surface is scarce or null in literature. Furthermore, the simulations for the next 100 years is that climate changes will continue (IPCC 2013), which raises the question what are the consequences to the estuarine Zooplankton community, with repercussions along the trophic food web.

1.1. Estuarine Ecosystems

Coastal marine areas represent approximately 4% of Earth's total land surface. They host about half of the world's human population and play a key contribution to global economic profits and ecological services (Mancinelli and Vizzini 2014; Barbier 2012; Selleslag *et al.*, 2012). Their long-term sustainability consists on the health of coastal ecosystems and the services that they provide, such as fisheries production, storm buffering and enhancement of water quality. However, at a global scale, degradation and loss of coastal ecosystems on the past decades have been intense (Duarte *et al.*, 2014). The effects of coastal degradation (e.g., loss of habitats and biodiversity, fishery decline, and an overall decrease in the life quality of local populations at social and economic level) are generally identifiable; conversely, there are often multiple, simultaneous causes of degradation, as several human-related pressures such as, overfishing, urban and industrial pollution, overlap their effects on ecosystems (Boesch *et al.*, 2001;Lotze *et al.*, 2006). Moreover, the hydrological and ecological complexity of estuarine and coastal systems makes them extremely susceptible to stressors, such as to global changes and extreme climate events, whose effects may combine with other direct and indirect factors. The increase in the frequency and magnitude of flooding events is expected to increase the flow of nutrients and chemicals into aquatic systems, which may have severe repercussions to the aquatic communities and, at a worst scenario, to the trophic food web (Mancinelli and Vizzini 2014).

Estuaries are located at the interface between the continental and marine domains and represent a complex mosaic of different habitats. These systems are characterized by a semiclosed water body with free connection to the open sea, where occurs seawater dilutions, contributing to a salinity gradient along the estuarine system. Estuaries are characteristically dynamics exhibiting a high degree of temporal and spatial variability in environmental conditions. Indeed, estuaries are subject to multiple environmental stressors and a major site for problems associated with inorganic and organic contaminants. A wide biological variety characterizes these ecosystems, with a high primary production as well as conditions more favorable to the biological development, than in the rivers and in the oceans.

Due to favorable conditions of the estuarine ecosystems, there are an intensification of the coastal activity and therefore an increase of the stress on these ecosystems. With the intensification of the coastal activity, we observe an intense urbanization of coastal areas, and an intensive agricultural activity of the fields in the surrounding areas of aquatic systems, with an intensive usage of pollutants and fertilizers (McCarthy *et al.,* 2007; Samelling *et al.,* 2013). In response to the behavior set of the maritime and fluvial forces, estuaries are exposed to a wide variety of compounds, such as pesticides, metals, oils, pollutants from industries and navigation ports (Macedo *et al.,* 2005). Estuarine ecosystems are very important and productive systems, they support a great variety of life resources, however these resources are extremely sensitive to the adverse effects of the several pollutants, from the affluent rivers and drainage of the surrounding farms, discharged to the estuaries (McCarthy *et al.,* 2007; Samelling *et al.,* 2013). The contaminants, mainly the persistent, tend to accumulate on the environmental and on the organisms, inducing exchanges on chemical characteristics of water quality and biological communities (Macedo *et al.,* 2005; Cardoso *et al.,* 2004).

The growth of primary producers such as macroalgae and phytoplankton are stimulated by a great entrance of nutrients from industrial, agricultural and urban effluents, due to the particular characteristics of these ecosystems (shallow depth and reduced water exchange), occurring algae blooms, one of the most frequent symptoms / consequences of the eutrophication. The main pressures of anthropogenic activities in estuarine ecosystems results in habitat loss, overexploitation of resources (due to overpopulation) and chemical and organic pollution (mainly from an extensive agricultural and industrial activities), resulting on topographic and hydrodynamic exchanges of the riverbed, increase of limiting nutrients concentration and water turbidity (Macedo *et al.,* 2005; Cardoso *et al.,* 2004).

1.2 The Mondego estuary - General Characterization

The Mondego estuary is located on the Atlantic Portuguese coast, near the city of Figueira da Foz (40º08´N, 8º50´W), it is a small mesotidal system with an area of 8.6 km^2 divided in two arms, north and south, separated at 7 km from the shore joining again near the mouth by an alluvium-formed island (Figure 1). Each arm presents different hydrological characteristics. The north arm is deeper (4-8 m during high tide with a range of 1 to 3 m), it has a low residence time (<1day) and it is the main navigation channel. The south arm, on opposition to the north, is shallower (2-4 m deep during the high tide with a range of 1 to 3 m), it has higher residence time (2-8 days) and it was largely silted up, especially on the upstream areas, until 2006. The flow of water in the south arm is mostly due to tidal cycle and the freshwater input comes from a small tributary, the Pranto River, which is controlled by a sluice. The south arm is affected by several human activities (direct and indirect) such as high input of nutrients from agricultural fields and aquacultures, pollutants from industrial and domestic discharges and the economic growth of the region over the years. Until 1998, the south arm was almost silted up in the innermost areas, and the river outflow occurred mainly via the north arm. Therefore, water circulation was here mostly dependent on tides and on the freshwater input from the Pranto River, a small tributary with a flow controlled by a sluice, which was regulated according to the water level of rice fields in the Mondego Valley. The main causes that alter the salinity of the waters of the estuary are related to high intensity precipitation over small periods of time, discharge of freshwater from the dams, and runoffs from the increasing eroded land (Lillebo et al., 2005). Drought episodes can also alter the salinity of the waters, due to evaporation and sodium chloride precipitation

1.3 Anthropogenic Pressures

Mondego estuary, like other estuaries, is under a strong anthropogenic pressure. Added to the port, beach and industrial activities and the exploitation of marine resources, the rice and corn cultures are the most relevant intensive agricultural activities in the Mondego valley, originating a significant input of nutrients and pesticides on estuarine waters, by leaching of surrounding agricultural farms, culminating on the ecosystem eutrophication. The eutrophication process in the Mondego estuary has led to the decrease of the global environmental quality of the estuary, as well as the degradation of water quality and turbidity increase, since the life quality and populations support depend on the conservation of the natural conditions of aquatic ecosystems and mitigation of negative impacts from utilization of water resources like receptors of point (industrial and domestic) and diffuse (agriculture and aquiculture) discharges, responsible for the progressive eutrophication of fluvial and estuarine ecosystems and consequent exchange of the trophic structure. Pesticides used in agricultural activity have been found both on surface and ground waters. This contamination may have (eco)toxicological effects to the aquatic flora and fauna and consequently to the human health (Cerejeira 2003).

1.4 Climate changes

As a consequence of climate change, flood and drought events are increasing in frequency throughout the world. There is a great concern about the impacts of climate changes worldwide due to the severe effects it may have in the environmental and to the aquatic communities. Recently Intergovernmental Panel on Climate Change report predicts the occurrence, at the next 100 years, of changes in salinity seawater, raise in temperature and water acidification, with the estuarine and coastal environments being the major affected areas (IPCC, 2014). It is known stressors affect the organisms' physiological conditions with some organisms adapted to some environmental changes. For instance, some works conducted to examine the response zooplankton community, and in more detailed the Copepoda community, of the Mondego estuary to the climatic variability (extreme drought) revealed the extreme drought event in 2005 was responsible for a higher dominance of marine species that remain along the next regular climatic years (2006 and 2007) (Marques et al., 2007; Gonçalves et al. 2012). Zooplankton community, mainly Copepoda assemblages presented a clear seasonal pattern that superimposed to the inter-annual variability. A more recent study (Marques et al. 2017) examined the effects of climate variations to plankton communities over the period 2003 to 2012. The most relevant change was observed in 2008 with a conspicuous increase in marine organisms mainly gelatinous zooplankton and small-sized copepods (*Oithona plumifera*, *Clausocalanus arcuicornis*, *Penilia avirostris*), with implications to the structure of the Mondego zooplankton community.

1.5 Zooplankton

This work study the Zooplankton community from the Mondego estuary, a southern European ecosystem. The Zooplankton that consists of a set of planktonic organisms that have no photosynthetic ability and live dispersed in the water column, with minimal swimming ability are largely dragged by ocean currents or the water of a river. Plankton, mainly zooplankton, is very sensitive to variations of biotic (e.g. predators, competition) and abiotic conditions (e.g. temperature, salinity, food quantity and quality) (Gonçalves et al., 2010 a, b; 2012 a, b, c). The variation of these factors may generate changes and adaptations that will be imprinted in the community composition. This can go from the low fixation of calcium making the exoskeleton of Crustaceans weaker or deformed, which can lead to the death of the organism (Rabalais et al., 2010), to the intolerance to the salinity rates which may change completely the planktonic community (Gonçalves et al., 2010 a, b; 2012 a, b, c) potentially harming the ecosystem. In estuarine ecosystems, the life – cycles of zooplanktonic organisms are related with environmental factors, since there are highly affected by those factors, mainly temperature and salinity (Vieira et al 2015). Several studies showed the importance of Zooplankton as a good indicator of environmental changes in the coastal ecosystems (Falcão et al., 2012, Tomczak et al., 2013, Vieira et al 2015). Zooplankton has a crucial role in marine/estuarine ecosystems being a link between primary producers and the higher trophic levels. The structure of the community and its diversity is a determinant factor for the support of pelagic food webs, since the presence of key taxa is vital for the survival of some phases of zooplanktivorous fish, thus supporting higher trophic levels (Gonçalves et al., 2010 a, b; 2012 a, b, c, Vieira et al 2015).

1.6 Main objectives

At the Mondego estuary was reported changes in the community during extreme climatic events such as droughts (Gonçalves et al 2012). During this event there an advance in the estuary of the marine/estuarine populations, making then present in the whole estuary (Gonçalves et al 2012, Primo et al 2012). Also it has been well reported the vertical dynamic in the mouth of the estuary (Gonçalves et al 2012). Although, studies about the influence of flood events in the Zooplankton community from the bottom and surface in whole estuary is null in literature. Indeed only the surface community was described for the whole estuary (Gonçalves et al., 2010 a, b; 2012 a, b, c), the vertical dynamics and the bottom community were only characterized for the entrance of the estuary, and so far it was not described for the whole estuary. By this, the present study aims to determine the influence of climatic events in the zooplankton communities from the bottom and surface along the Mondego estuary.

2. Materials and Methods

2.1. Sampling procedures and *in situ* measurements

Samples were taken monthly during high tide, from May 2012 to May 2014, at six stations located over both arms of the estuary: St 2 – mouth station; St 5 and St 9 - southern arm stations, St 12, St 18 and St 23 - northern arm stations (Figure 1), in order to have representative area coverage of the system, along the salinity gradient. The surface samples were collected by sub-surface tows (1 m below the surface) and the bottom samples were collected 1 m above de bottom, with a 335 µm mesh net (mouth diameter 0.30 m), equipped with a Hydro-Bios flow meter mounted in the mouth to estimate the volume of water filtered by the net. Sampling was performed at spring tides with few exceptions due to logistic constrains. After collection, samples were fixed in 4% buffered formaldehyde. Simultaneously, environmental parameters such as water temperature (ºC), salinity (WTW Cond 330i, WTW Wissenschaftlich - Technischewerkstätten, Germany), dissolved oxygen (DO, (WTW OXI 330i, mg.L^{-1}), pH were measured *in situ* and the water transparency, considered as maximum visible depth, was measured with a Secchi disc (m).

2.2. Laboratorial procedures

Water samples were taken in each sampling station for determination in laboratory of chlorophyll *a* (Chl *a*, mg.m^{-3}), nutrient concentrations (nitrates, phosphates, ammonia, soluble silica – mg.L^{-1}) and total suspended solids (TSS, mg.L^{-1}) according to standard protocols (for further information please see Gonçalves et al. 2010 a, b). Samples were filtered using GF/C filters and stored frozen at -18 ºC until further analysis. Zooplankton sub-samples were obtained for numerical abundance using a Folsom plankton splitter. At each sub-sample a minimum of 500 individuals were counted. The organisms were counted (number of organisms m^{-3}) and identified to the lowest possible *taxon* using a microscope and magnifying glass.

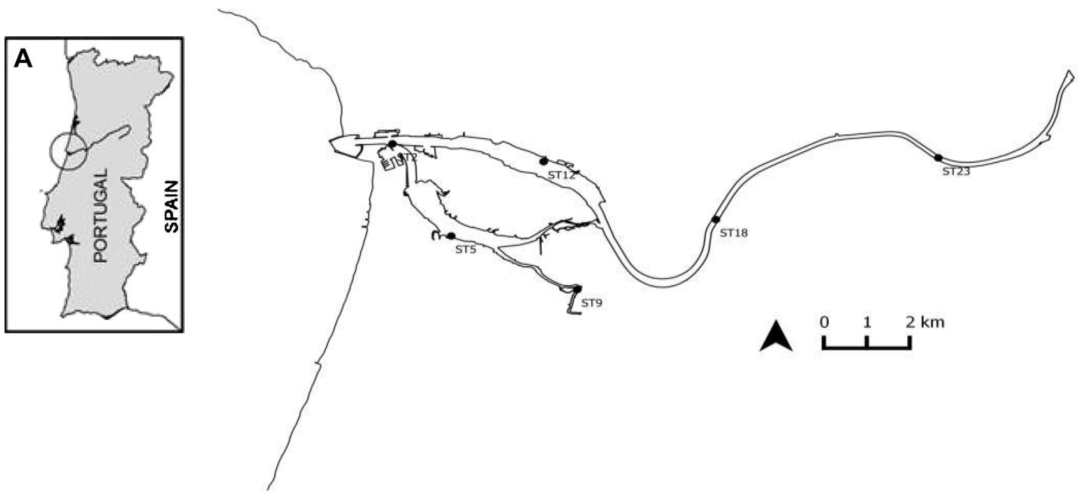

Figure 1. Location of the Mondego estuary on the western coast of Portugal and the six sampling stations (St 2 - Mouth station, St 5 and St 9- south arm stations, St 12, St 18 and St 23- north arm stations).

2.3. Data Analysis

For each sample, the number of *taxa* was counted, and the specific diversity (H') was calculated with the Shannon-Wiener equation while the equitability was determined by the Pielou's evenness index (E) (Washington, 1984).

Proceeding with Primer 6 to run the statistical analysis, a PCA and ANOSIM were performed to compare biological and environmental parameters and assess similarities and dissimilarities between groups. A reduce data analysis were carried out using CANOCO 4.5. Biologic data were transformed by square root, the environmental parameters were transformed by logarithmic base 10. Both matrices, the taxonomic groups and environmental parameters, respectively, and rows of the seasonal data, which were estimated by averaging the monthly values from each station, to identify the relationship between species distribution and environmental factors.

3. Results

3.1. Environmental parameters

In the last decade drastic differences have been recorded when compared to the general climate patterns for the period of 1971-2000 (Gonçalves *et al.*, 2012). The year 2012 was characterized by a severe and extreme drought event that was maintained all over the year with higher intensity in late winter and early spring. Anomaly precipitation values on the following months were recorded for the region of the Mondego estuary: January (-96.9 mm); February (-97.9 mm); March (-40.4 mm) and April (-33 mm), which recorded precipitation values much lower than the climatological regime for the centre of Portugal in 1971-2000 (http://snirh.apaambiente.pt). In fact, the year 2012 was considered the 8th driest year of the last 82 years, with the months of late winter and early spring being the driest since 1931.

The year of 2014 was characterized by an extreme flood event, also considered as a very rainy year, with an annual average of precipitation significantly higher than the normal registered at 1971-2000, with a deviation of +216.1 mm, standing out like the rainiest year of the last 25 years (Do ambiente, 2015), with February being the wettest month of the last 35 years. For the Mondego estuary basin positive anomaly precipitation values were observed at the following months: January (+ 44.3 mm); February (+ 105 mm); October (+ 98.2 mm) and November (+ 109.4 mm), which recorded precipitation values much higher than the climatological normal for central Portugal in 1971-2000 (http://snirh.apaambiente.pt).

As expected, the environmental parameters, namely temperature and salinity, showed a distinct pattern when winter and summer are compared (Table 1; Figures 2-3). Winter is marked by a reduction in salinity and temperature values that occurs by an increase in the input of freshwater, mainly in winter of 2014 where a strong rainfall was registered causing floods events. In winter season was registered variations in oxygen, phosphate, nitrite and nitrate; in summer, the influence by the sea water counterbalanced the winter lower values of salinity.

In this case, the variability comes from Chlorophyll a concentration, TSS, temperature, salinity, turbidity and ammonia. Between spring and autumn there were not registered significant differences. One of the main stressors for the zooplankton was the gradient of salinity that suffers major reductions during the winter and spring due to the increase of freshwater uptake from the river and rain; However, for st2 (Figure 2) and comparing the bottom with the surface the values of salinity, during the whole period of the study, were almost the same. This occurs because St 2 is near the mouth of the estuary where the sea water has a high influence.

The PO_4 values has and increase during the winter, NO_2 and NH_4 did not differ their values during the period of the study. NO_3 reaches its peak during the autumn of 2012 then returns to its regular values.

Figure 2: Variation of salinity (‰) and temperature (ºC) for each station during the study period, for the salinity surface (green) and bottom (purple) and for the temperature surface (blue) and bottom (red).

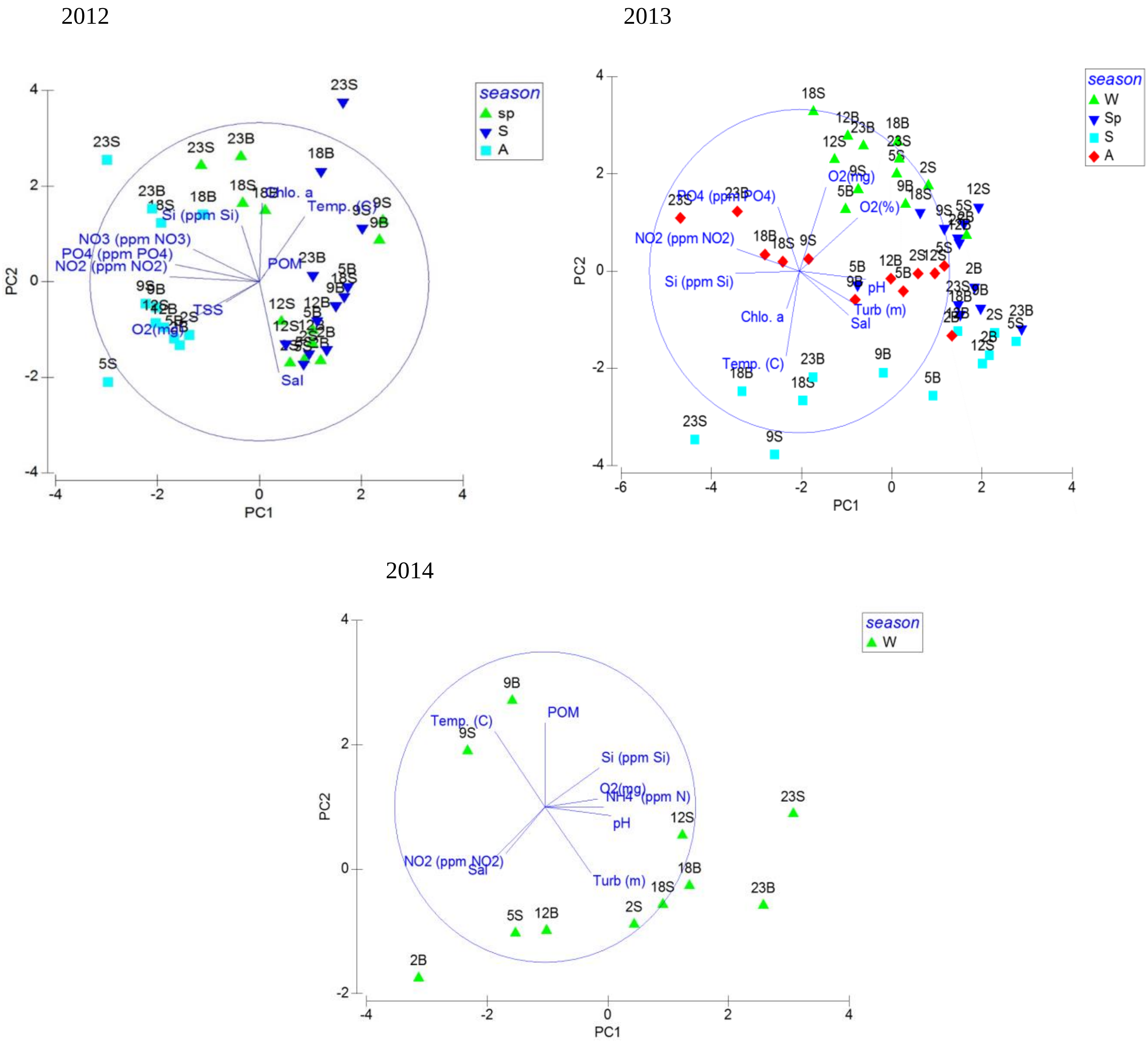

Figure 3. Result of the PCA for each year (2012, 2013, 2014), to each season (Sp – Spring; S – Summer; A – Autumn; W – Winter); for each station is represented the surface sample (S) and the bottom samples (B).

Table 1. Physico-chemical data, indicated as seasonal means values, measured during field campaigns in the Mondego estuary, from May 2012 to March 2014 in Stations St2, St5, St9, St12, St18 and St23.

Station	Season	Year	sample	Temp. (ºC)	Sal (‰)	pH	O_2(%)	O_2(mg)	cond. (µS/cm)	Turb (m)	Dept (m)	Si (ppm Si)	PO_4 (ppm PO	NO_2 (ppm NO	NO_3 (ppm NO	NH_4 (ppm N)	Chlo. a	TSS (g.L^{-1})	POM (g.L^{-1})
St2	spring	2012	Surface	16.950	31.215	7.310	92.900	7.430	40302.500	1.900	11.400	0.883	0.122	0.051	0.764	0.036	3.538	0.013	0.003
			Bottom	16.400	34.300	7.620	74.600	5.925	43541.000	1.900	11.400	0.274	0.191	0.057	0.685	0.078	4.460	0.018	0.005
		2013	Surface	16.600	16.155	7.950	92.350	8.265	21712.500	2.400	9.600	1.963	0.667	0.155	0.978	0.050	6.329	0.014	0.003
			Bottom	15.650	35.345	8.090	80.200	6.420	43955.500	2.400	9.600	1.711	0.934	0.274	0.766	0.055	5.194	0.041	0.006
		2014	Surface	15.850	30.180	7.540	98.550	8.145	38454.500	2.500	12.000	0.929	0.140	0.090	0.719	0.042	0.381	0.008	0.002
	Summer	2012	Surface	16.900	34.530	8.040	77.300	6.105	44118.500	2.450	9.100	0.274	0.058	0.115	1.532	0.011	5.072	0.019	0.003
			Bottom	16.700	35.595	8.075	67.350	5.310	45263.000	2.450	9.100	0.163	0.102	0.112	1.253	0.062	5.511	0.026	0.004
		2013	Surface	18.850	33.290	8.065	89.800	6.825	44786.000	1.650	9.300	1.431	0.097	0.082	0.253	0.115	6.624	0.016	0.004
			Bottom	17.450	35.335	8.060	76.450	5.865	45837.000	1.650	9.300	0.401	0.143	0.088	0.220	0.115	5.549	0.029	0.004
	Autum	2012	Surface	16.850	30.123	7.960	91.450	7.470	43649.500	1.400	8.200	1.758	0.254	0.486	2.989	0.083	1.792	0.023	0.004
			Bottom	17.150	35.187	8.010	86.750	7.050	44997.000	1.400	8.200	1.200	0.477	0.660	2.385	0.085	2.625	0.025	0.004
		2013	Surface	16.750	32.505	7.960	87.100	6.970	41699.500	1.150	9.750	3.461	0.448	0.148	0.846	0.120	2.129	0.025	0.004
			Bottom	16.750	35.345	7.945	71.600	5.645	44971.000	1.150	9.750	0.306	0.284	0.137	0.358	0.080	3.076	0.068	0.009
	winter	2013	Surface	12.850	7.530	7.950	86.600	8.805	9929.000	1.050	10.250	1.316	0.373	0.041	0.973	0.026	1.393	0.026	0.003
			Bottom	12.950	34.415	7.950	80.800	6.925	40294.000	1.050	10.000	0.149	0.827	0.171	0.331	0.042	1.650	0.034	0.004
		2014	Surface	12.850	2.480	7.835	92.150	9.655	3588.000	1.250	12.100	2.078	0.382	0.193	1.322	0.025	1.435	0.009	0.002
			Bottom	13.350	32.355	7.100	81.400	6.650	38568.000	1.250	12.100	1.298	0.744	0.187	1.251	0.037	2.850	0.035	0.004
St5	spring	2012	Surface	16.950	32.480	8.175	83.900	6.675	41871.000	1.650	3.000	0.929	0.143	0.083	0.516	0.057	3.332	0.022	0.004
			Bottom	16.850	22.835	8.455	76.000	6.135	30394.500	1.650	3.000	0.634	0.177	0.084	0.253	0.082	4.553	0.024	0.004
		2013	Surface	16.600	24.210	8.085	93.500	7.935	32131.500	1.600	2.000	1.988	0.885	0.344	0.738	0.311	3.911	0.018	0.004
			Bottom	20.900	11.195	7.915	88.250	7.470	17363.500	0.450	3.600	1.488	0.728	0.169	1.492	0.056	25.867	0.035	0.007
		2014	Surface	15.950	32.145	7.580	91.950	7.515	40627.000	2.100	2.800	1.419	0.609	0.178	0.693	0.024	0.300	0.014	0.002
	Summer	2012	Surface	17.100	35.355	8.050	80.700	6.330	45432.500	1.500	1.500	0.165	0.103	0.104	1.447	0.077	4.030	0.023	0.003
			Bottom	20.250	33.125	7.925	66.850	5.115	46023.500	0.950	1.900	0.497	0.119	0.126	1.348	0.079	7.867	0.030	0.004
		2013	Surface	18.800	34.215	8.045	89.800	6.800	45810.500	2.150	2.150	1.143	0.095	0.099	0.088	0.100	5.786	0.021	0.004
			Bottom	21.700	30.950	7.995	77.050	5.495	44293.000	1.500	2.750	2.306	0.177	0.007	0.182	0.265	12.002	0.025	0.004
	Autum	2012	Surface	16.900	32.945	8.020	84.850	7.305	41962.000	0.900	2.450	0.756	0.463	0.586	3.460	0.080	1.921	0.162	0.003
			Bottom	17.050	29.112	8.005	75.700	6.645	42409.000	0.900	2.450	0.046	0.419	0.628	3.320	0.080	3.341	0.052	0.006
		2013	Surface	16.750	34.220	7.900	92.100	7.255	43799.000	1.050	2.450	2.227	0.385	0.151	0.661	0.120	2.129	0.024	0.004
			Bottom	16.500	30.695	7.920	78.750	6.470	39729.500	1.050	3.500	2.433	0.373	0.252	1.125	0.350	2.943	0.033	0.005
	winter	2013	Surface	11.950	19.355	7.930	84.950	8.030	23438.000	0.850	2.500	1.907	0.807	0.107	1.582	0.061	1.519	0.026	0.003
			Bottom	12.600	7.130	7.845	77.300	7.665	9667.000	0.550	3.500	1.836	0.497	0.084	1.815	0.079	9.018	0.023	0.006
		2014	Surface	12.950	13.645	7.120	90.300	8.865	17403.500	1.100	2.750	1.787	0.556	0.191	0.768	0.033	1.424	0.013	0.002

Table 1. Continued

St9	spring	2012	Surface	21.950	20.425	7.660	53.350	4.060	31178.000	0.500	3.500	1.862	0.174	0.061	0.832	0.073	4.877	0.020	0.003
			Bottom	20.950	22.060	8.090	48.750	3.795	31650.500	0.500	3.500	1.689	0.176	0.067	0.883	0.074	3.727	0.030	0.005
		2013	Surface	19.100	11.895	8.075	91.750	7.710	18187.450	0.800	4.750	0.322	0.862	0.348	0.664	0.048	6.930	0.027	0.004
			Bottom	16.850	30.315	8.110	80.500	6.510	39076.000	1.850	6.050	0.215	0.511	0.476	0.931	0.093	7.632	0.016	0.003
		2014	Surface	19.200	16.150	7.340	79.850	6.850	23435.500	0.800	3.150	3.632	0.411	0.269	1.924	0.037	1.662	0.026	0.004
	Summer	2012	Surface	22.700	30.915	7.835	67.400	4.285	45866.000	0.550	2.400	0.880	0.197	0.180	1.698	0.080	9.282	0.034	0.005
			Bottom	20.700	33.055	7.960	69.450	5.055	46219.500	0.400	3.700	0.404	0.152	0.296	1.127	0.094	3.692	0.028	0.003
		2013	Surface	25.500	26.730	7.600	52.650	3.670	42067.000	0.400	3.750	3.273	0.351	0.049	0.460	0.185	15.411	0.045	0.006
			Bottom	22.550	31.440	7.890	72.200	5.355	45704.000	1.250	4.600	2.907	0.422	0.053	0.717	0.110	5.861	0.038	0.005
	Autum	2012	Surface	19.500	28.100	7.865	81.300	6.745	40579.000	0.700	2.800	0.363	0.915	0.717	3.215	0.006	1.824	0.028	0.004
			Bottom	19.000	32.888	7.860	69.750	6.080	40412.500	0.700	2.800	1.272	0.773	0.774	2.853	0.012	1.794	0.065	0.008
		2013	Surface	16.900	28.420	7.720	71.350	5.875	37069.000	0.650	3.600	2.102	1.002	0.163	2.485	0.196	5.877	0.029	0.006
			Bottom	17.200	31.360	7.755	74.350	5.970	40792.000	0.700	4.400	3.303	0.474	0.142	0.935	0.165	2.956	0.037	0.005
	winter	2013	Surface	12.200	9.660	7.695	75.650	7.680	12415.000	0.650	4.850	0.615	1.201	0.124	0.744	0.076	14.949	0.036	0.005
			Bottom	13.000	17.515	8.010	84.250	8.135	20826.000	0.600	6.500	1.865	0.494	0.135	1.336	0.031	5.025	0.112	0.012
		2014	Surface	15.250	4.665	7.125	86.500	8.540	7051.500	0.600	4.850	2.091	0.632	0.210	0.834	0.054	13.321	0.029	0.010
			bottom	14.600	4.385	7.255	72.900	7.345	7046.250	0.600	4.850	2.595	0.884	0.125	1.300	0.026	14.458	0.030	0.013
St12	spring	2012	Surface	17.300	25.315	7.085	87.450	7.145	33669.500	1.400	6.100	1.695	0.183	0.051	1.120	0.059	4.074	0.010	0.002
			Bottom	16.800	33.450	8.115	75.650	6.005	42944.500	1.400	6.100	0.273	0.198	0.048	0.681	0.032	7.670	0.049	0.008
		2013	Surface	15.800	17.000	8.015	94.750	8.555	21949.950	2.000	5.950	0.213	0.895	0.489	1.109	0.099	7.997	0.021	0.004
			Bottom	19.350	11.060	8.170	93.950	8.165	16592.500	1.300	5.400	0.329	0.769	0.553	1.116	0.051	13.036	0.013	0.004
		2014	Surface	16.150	24.540	7.605	90.800	7.775	32975.000	1.450	4.750	1.668	0.476	0.140	2.275	0.037	0.414	0.015	0.002
	Summer	2012	Surface	17.300	33.895	8.040	70.800	5.565	43909.000	1.100	8.150	0.145	0.116	0.408	1.829	0.116	3.839	0.019	0.003
			Bottom	17.650	31.205	7.955	67.350	5.350	41484.000	1.300	7.900	0.445	0.200	0.044	1.620	0.174	5.817	0.038	0.005
		2013	Surface	18.800	33.205	7.955	78.050	6.045	44524.000	2.050	5.300	0.657	0.098	0.082	0.311	0.090	5.348	0.023	0.004
			Bottom	18.950	30.710	7.895	82.850	6.975	42233.500	1.600	5.150	1.267	0.131	0.087	0.451	0.085	6.987	0.018	0.004
	Autum	2012	Surface	16.800	30.840	8.045	79.667	6.557	40720.000	2.100	6.150	1.083	0.784	0.636	3.033	0.052	2.431	0.014	0.003
			Bottom	16.800	35.440	8.085	72.933	5.747	50444.500	2.100	6.150	1.119	0.912	0.675	2.657	0.127	2.245	0.027	0.004
		2013	Surface	16.750	33.820	7.905	86.600	6.825	43343.500	1.100	5.600	0.874	0.381	0.120	1.514	0.077	2.030	0.034	0.005
			Bottom	16.400	26.295	7.710	80.450	6.770	34599.500	1.100	5.450	2.744	0.408	0.130	0.461	0.090	2.501	0.035	0.005
	winter	2013	Surface	12.550	16.195	7.750	81.850	7.920	18950.350	0.600	5.600	2.581	1.029	0.519	1.973	0.069	2.493	0.017	0.003
			Bottom	12.200	0.100	7.625	86.700	9.330	162.950	0.450	4.600	1.857	0.579	0.598	1.328	0.055	2.408	0.107	0.011
		2014	Surface	13.100	0.620	7.810	93.950	9.850	971.400	1.000	5.750	2.170	0.663	0.096	1.810	0.056	1.533	0.006	0.002
			bottom	12.550	16.065	7.385	79.000	7.700	19479.400	1.000	5.750	2.469	0.795	0.193	2.490	0.045	1.499	0.014	0.002

Table 1. Continued

St18	spring	2012	Surface	17.550	15.075	8.005	64.150	5.450	20489.550	0.750	5.050	2.497	0.077	0.071	5.727	0.021	7.963	0.014	0.003
			Bottom	17.600	15.250	8.000	59.100	5.065	20797.550	0.750	5.050	2.457	0.095	0.073	4.517	0.041	7.967	0.031	0.004
		2013	Surface	16.150	9.050	7.820	90.050	8.470	12813.200	1.350	5.150	0.499	0.774	0.248	0.953	0.039	11.625	0.012	0.003
			Bottom	18.850	15.405	8.005	86.500	7.370	20587.350	2.300	6.700	0.490	0.329	0.224	1.221	0.062	15.648	0.015	0.003
		2014	Surface	17.450	4.495	8.150	88.400	8.290	6792.000	0.900	4.650	3.365	0.532	0.351	2.523	0.039	0.831	0.029	0.006
	Summer	2012	Surface	19.250	29.685	7.925	64.050	5.000	40676.000	0.950	5.050	1.013	0.147	0.014	1.526	0.162	4.229	0.029	0.004
			Bottom	21.150	15.720	7.615	66.250	5.345	21312.500	0.800	5.450	1.749	0.159	0.101	2.069	0.140	15.103	0.034	0.006
		2013	Surface	23.700	14.060	7.705	60.000	4.660	22707.500	1.250	4.450	2.769	0.322	0.163	1.314	0.090	11.287	0.024	0.003
			Bottom	24.300	8.355	7.570	56.500	4.525	13748.500	0.900	4.150	3.186	0.373	0.171	1.905	0.090	11.808	0.021	0.003
	Autum	2012	Surface	17.550	19.465	7.835	75.233	6.375	31685.500	1.650	5.000	4.522	0.611	0.681	2.402	0.030	5.219	0.018	0.003
			Bottom	17.400	20.320	7.850	67.350	5.682	33805.000	1.650	5.000	3.463	0.535	0.484	2.637	0.066	9.301	0.025	0.004
		2013	Surface	16.700	18.155	7.500	66.200	5.725	24282.500	0.950	4.750	2.766	0.951	0.112	1.708	0.091	3.191	0.020	0.004
			Bottom	16.000	7.665	7.470	68.000	6.585	11258.000	1.000	4.400	3.800	0.527	0.164	1.850	0.085	3.849	0.056	0.007
	winter	2013	Surface	12.000	0.085	7.770	82.000	8.895	135.650	0.550	4.100	3.143	1.239	0.562	1.751	0.081	3.635	0.014	0.004
			Bottom	12.650	3.480	7.650	94.450	9.860	4655.650	0.600	9.250	1.573	0.399	0.060	0.656	0.011	2.103	0.028	0.005
		2014	Surface	12.400	0.080	7.685	91.600	9.850	126.600	1.050	4.600	2.032	0.669	0.173	1.424	0.078	1.111	0.013	0.002
			bottom	12.500	0.080	7.690	79.650	8.525	129.700	1.050	4.600	2.806	0.710	0.174	2.293	0.121	1.369	0.010	0.001
St23	spring	2012	Surface	20.200	2.515	6.485	70.250	6.445	4402.300	0.750	5.100	2.944	0.265	0.533	3.771	0.055	6.872	0.010	0.002
			Bottom	19.850	3.410	6.035	59.250	5.600	5768.450	0.750	5.100	2.847	0.350	0.586	1.560	0.059	11.186	0.021	0.004
		2013	Surface	18.200	17.760	7.980	85.000	7.185	22725.750	2.300	6.700	0.463	0.516	0.276	1.096	0.066	14.580	0.020	0.004
			Bottom	18.800	32.820	8.115	89.000	6.845	44074.000	2.000	5.800	0.462	0.242	0.098	0.202	0.092	6.050	0.020	0.004
		2014	Surface	17.400	0.155	8.260	88.150	8.505	271.650	1.250	5.500	2.094	0.161	0.303	1.638	0.019	0.895	0.392	0.003
	Summer	2012	Surface	23.700	4.695	7.420	57.000	4.685	7619.500	0.700	5.650	1.315	0.251	0.075	2.188	0.051	17.573	0.033	0.005
			Bottom	19.850	22.025	7.710	74.650	5.960	29633.000	0.950	6.900	0.868	0.237	0.111	1.497	0.067	3.958	0.029	0.012
		2013	Surface	25.700	0.280	7.760	42.300	3.460	242.920	0.750	3.550	4.015	0.260	0.377	2.446	0.245	14.774	0.020	0.003
			Bottom	23.600	16.050	7.760	62.100	4.775	21841.550	1.250	6.500	2.258	0.371	0.116	1.863	0.090	5.537	0.015	0.002
	Autum	2012	Surface	18.400	0.415	7.320	74.517	6.852	1000.500	0.700	3.750	0.142	1.116	0.801	2.789	0.061	18.601	0.030	0.005
			Bottom	16.650	4.425	7.775	75.450	7.280	5955.500	0.900	6.000	0.504	1.068	0.214	2.251	0.063	16.552	0.039	0.005
		2013	Surface	16.200	0.440	7.665	63.300	6.335	750.500	0.850	4.200	5.363	1.045	0.331	3.822	0.084	6.949	0.021	0.004
			Bottom	15.500	0.460	7.630	72.350	7.440	783.500	0.850	8.100	4.536	0.681	0.334	2.971	0.100	7.616	0.016	0.003
	winter	2013	Surface	12.100	17.085	7.950	82.400	8.050	19947.800	0.600	9.250	1.965	1.185	0.452	0.636	0.043	2.545	0.027	0.005
			Bottom	12.900	7.830	7.730	92.700	9.145	9708.500	1.050	5.900	3.300	0.727	0.203	1.509	0.026	2.414	0.012	0.003
		2014	Surface	12.250	0.055	7.780	94.950	10.245	88.450	1.550	5.500	2.946	0.715	0.134	1.554	0.133	0.813	0.036	0.017
			bottom	12.300	0.055	7.680	79.050	8.495	87.850	1.550	5.500	3.287	0.649	0.142	1.280	0.138	0.436	0.011	0.001

3.2. Zooplankton composition and seasonal distribution

During this study, it was identified 91 species with the most representative groups being Cnidaria, Copepoda, Cladocera, Decapoda, and Mysidacea (table 2). Species with the life stages nauplii, juveniles and adults were also identified.

Table 2. List of species identified during the period of study in the Mondego estuary. n. id. - not identified.

	Acartia clausi
	Clausocalanus arcuicornis
	Paracalanus parvus
	Calanus helgolandicus
	Temora longicornis
	Centropages chierchaea
	Oithona plumifera
	Copepodite *(Calanus helgolandicus)*
	Isias clavipes
	Corycaeus anglicus
	Copepodite *(Centropages typicus)*
	Euterpina acutifrons
Copepoda	*Paranynochampus nanus*
	Nauplii copepoda
	Centropages typicus
	Oithona sp.
	Acartia tonsa
	Acanthocyclops robustus
	Copepodite *(Centropages chierchaea)*
	Calanipeda aquae dulcis
	Eurytemora velox
	Paracalanus sp.
	Copidodiaptomus numidicus
	Copepodite *(Calanus helgolandicus)*
	Oithona nana

	Diaptomus castor
	Copepoda n. id.
	Centropages sp.
	Euterpina acutifrons
	Pseudocalanus parvus
	Alteutha interrupta
	Sapphirina sp.
	Corycaeus sp.
Decapoda	*Rhithropanopeus harrisii*
	Carcinus maenas
	Pachygrapsus marmoratus
	Upogebia (zoea)
	Upogebia (zoea I)
	Upogebia affinis
	Palaemon
	Pisidia longicornis (zoea I)
	Porcellana platycheles
	Carcinus maenas (megalopa)
Cladocera	*Daphnia* sp.
	Evadne spinifera
	Daphnia longispina
	Penilia dana
	Bosmina sp.
	Penilia avirostris
	Podon polyphomoides
	Podon leukarti
	Evadne nordmanni
	Penilia sp.
Mysidacea	*Praunus flexuosus*
	Mesopodopsis slabberi
	Gastrosaccus santus
Amphipoda	*Echinogammarus marinus*
	Echinogammarus sp.

	Gammaridea sp.
Isopoda	*Paragnathia formica*
Ostracoda	*Ostracoda* n.id.
Cnidaria	*Muggiaea atlantica*
	Diphys sp.
	Sarsia tubulosa
	Solmaris corona
	Obelia sp.
	Lizzia blondina
	Podocoryne minima
	Mitrocomella sp.
	Sarsia sp.
	Liriope tetraphylla
	Margelopsis haeckeli
	Sarsia prolifera
	Zanclea costata
Chaetognata	*Sagitta friderici*
Doliolidae	*Doloilum* sp.
Cirripedia	*Cypris* cirripedia
	Nauplii cirripedia
Mollusca	Veliger bivalvia
	Veliger gastropod
Echinodermata	*Ophiopluteus* sp.
	Larva Ophiuroidea
Ichthyoplankton	Larva n. id.
	Pomatoschistus (larva)
Bryozoa	Larva Cyphonautes
Polychaeta	Larva Polychaeta n. id.
	Larva Sabellariidae
	Larva polynoid (Polychaeta)
	Spionidae (Trocophora)
	Magelonidae (larvae)

The highest number of *taxa* was observed during summer 2012 at stations near the mouth of estuary (St 2 and St 5) in the surface samples (Figure 4). At stations 18 and 23, during summer and autumn of 2012 and spring and autumn of 2013, the number of *taxa* was higher near the bottom. The lowest values were observed during winter without a defined distribution pattern.

During the sampling period, no pattern of variation of diversity was observed. Despite this, lower values of H' were observed at upper stations, during spring and autumn 2013, when compared with other sampling sites. The highest values of H' was obtained during summer of 2012 along the estuary.

The highest value of evenness (E) was obtained during winter 2014, at the bottom of St 23, and the lowest values were observed at the bottom of stations 5 (winter 2012) and 9 (autumn 2012) and near the surface of station 9 (autumn 2013 and winter 2014). These lower values are defined by the dominance of some *taxa*, expressed by higher abundances.

During the study period, it was noticed different distribution of the zooplankton community from the surface and bottom to each season and station. During the Spring 2012 season (Figure 5) some species (e.g. *Acartia clausi, A. tonsa*) are present on both bottom and surface samples but other species showed a preference (> 5% of abundance) of either bottom (e.g. *Palaemon* sp.) or surface (e.g. larva *Pomatochistus*), (p>5%). Considering the bottom samples, the station St 9 showed a clear dominance of *A. tonsa* in average abundance and a higher number of species when compared with the surface (Figure 5). In opposition, for the station St 2, almost all species are present both in the surface and bottom, except *Paleamon* sp. and *Pachygrapsus marmoratus* (bottom) and *Upogebia affinis* and *Pomatochistus* larva (surface) (Figure 5). *Temora longicornis* is the most abundant species at the surface and, in the bottom, share this position with *Carcinus maenas* and *Isias clavipes*.

At station St 5, the surface is dominated by *Acartia clausi*, while the bottom is represented by *A. clausi, A. tonsa, and veliger* of gastropods. St 12 showed a similar pattern: *A. clausi* dominated the community on both bottom and surface, having a more diverse community on the bottom with higher abundances (Figure 5). At upstream of the estuary, and following the pattern of the other stations, St 18 showed a higher number of species on the bottom that is described by *Copidodiaptomus numidicus, Daphnia* sp., *Bosmina* sp., *Acanthocyclopes robustus*. At the surface, the species *Diaptomus castor* dominated in abundance. At station St 23, *Acartia tonsa, Eurytemora* *velox*, *Acanthocyclops robustus*, *Copidodiaptomus numidicus* only occur at the bottom (Figure 5).

In general, the summer 2012 period showed low abundances, like last season (Figure 6). *A. tonsa* dominated the samples obtained in the bottom at stations St 5, St 9 and St 12. At station St 2, no dominant species were found, considering bottom and surface samples. The highest number of *taxa* were found at stations St 9 and St 23, for bottom samples. Comparing the last period with summer 2012, *A. tonsa* still dominates the community at station St 9 and, at station St 18, it was observed a change on the community composition (disappearance of freshwater species). The same pattern was observed at station St 23 with the presence of estuarine species (*e.g.*, *R. harrisii*, *Palaemon* sp.). This biological pattern was followed by the reduction in the freshwater input, due to the drought occurred during 2012, and the entrance of marine water in the estuary (29 ‰ of salinity registered at station St 18).

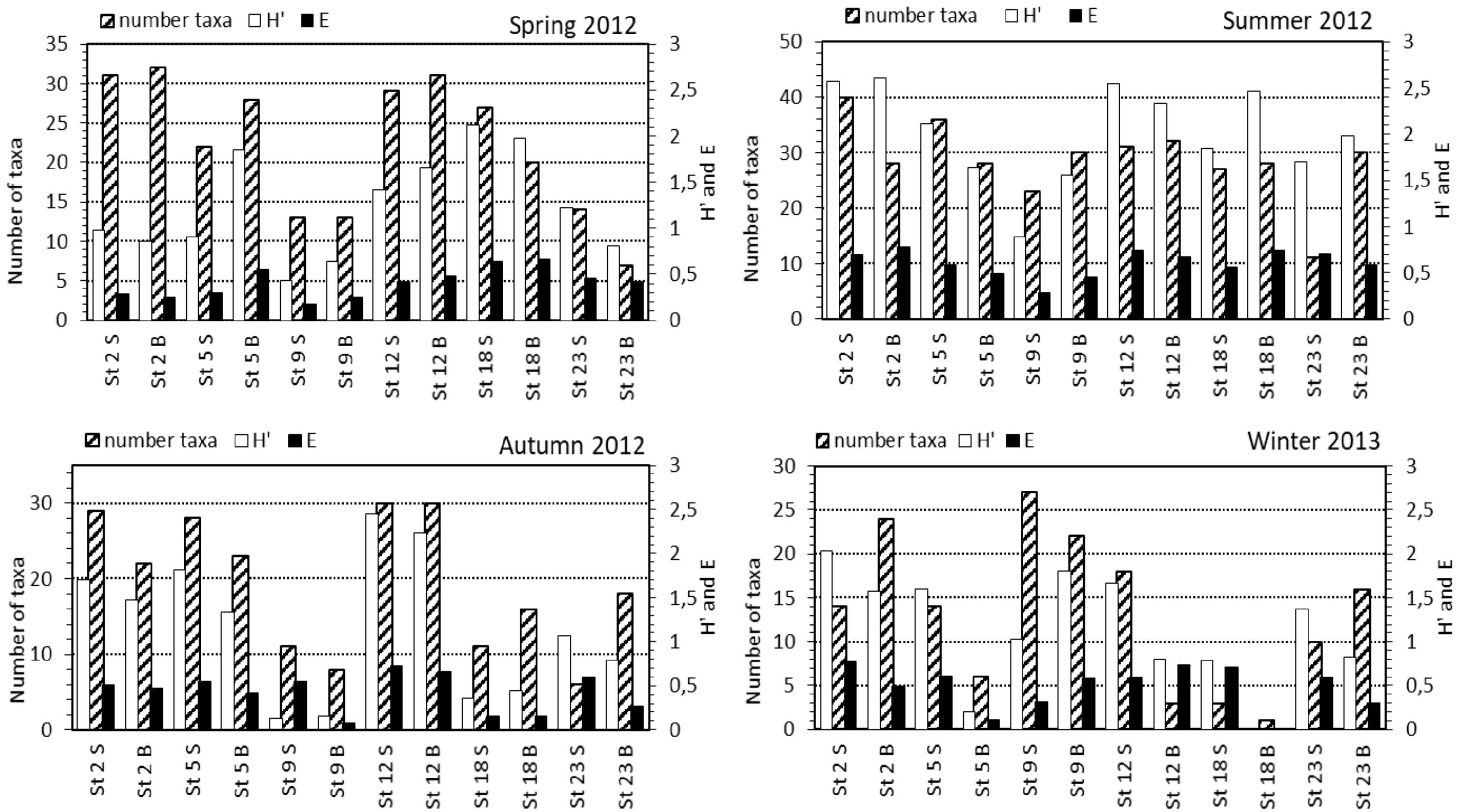

Figure 4: Variation of number of *taxa*, diversity (H’) and evenness (E) during the sampling period (from spring 2012 to winter 2014), at six sampling stations (St 2, St 5, St 9, St 12, St 18, St 23), at surface (S) and bottom (B), in the Mondego estuary. The dual yy axes presented different scales.

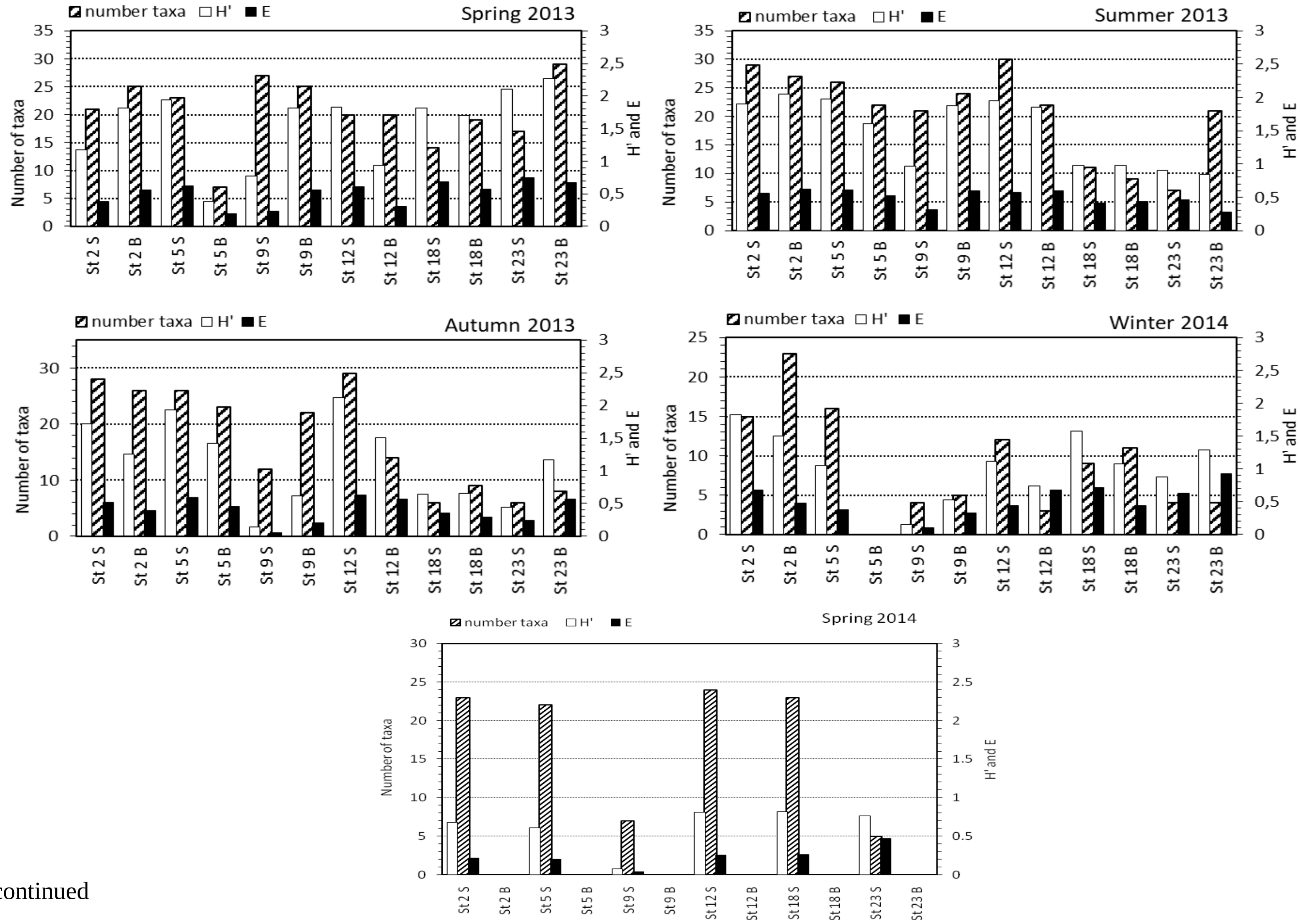

Figure 4: continued

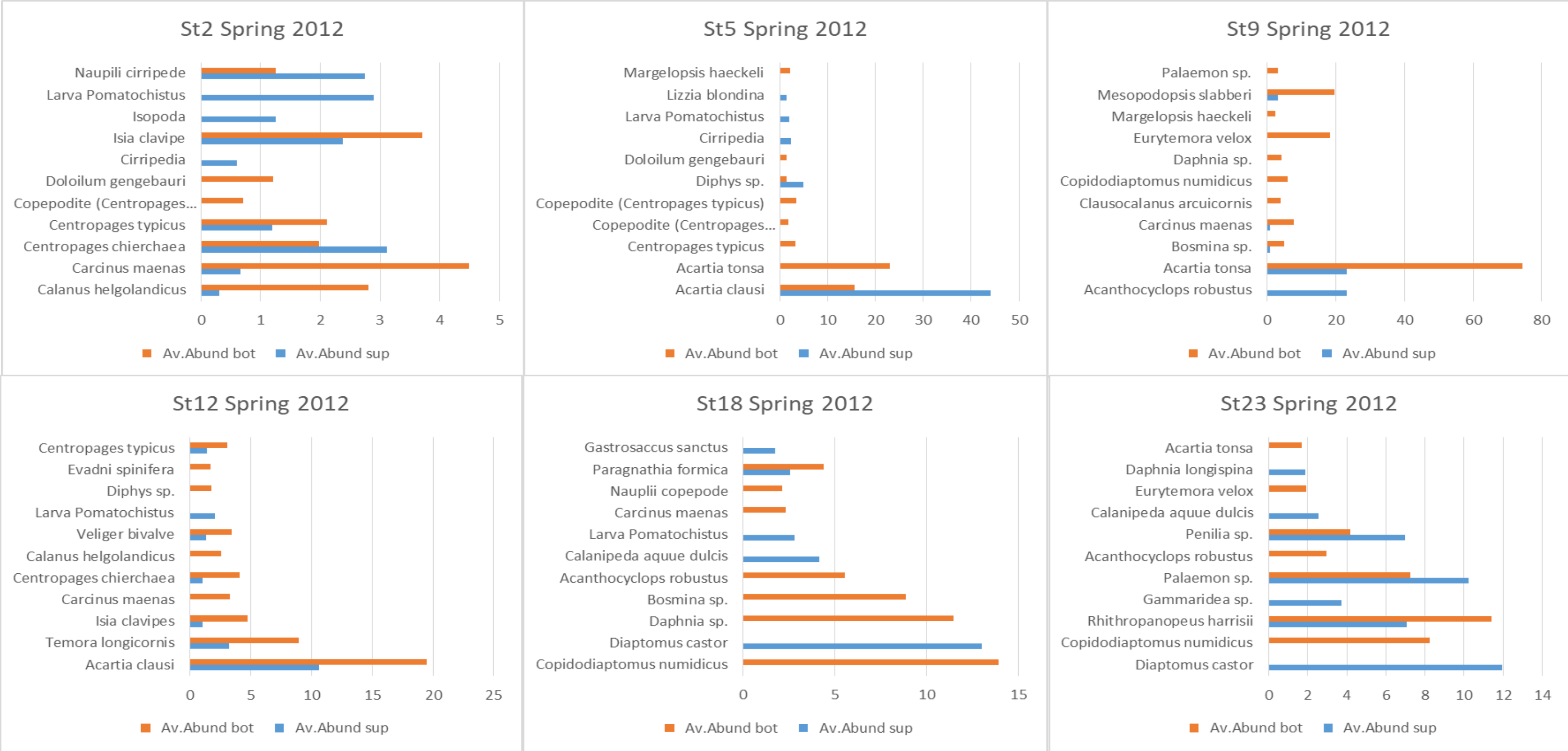

Figure 5: Average abundances for the community of zooplankton in the estuary of Mondego by station, during spring of 2012. Av. Abund bot - Average abundances at the bottom; Av. Abund sup - Average abundances at the surface. X axis with different scales.

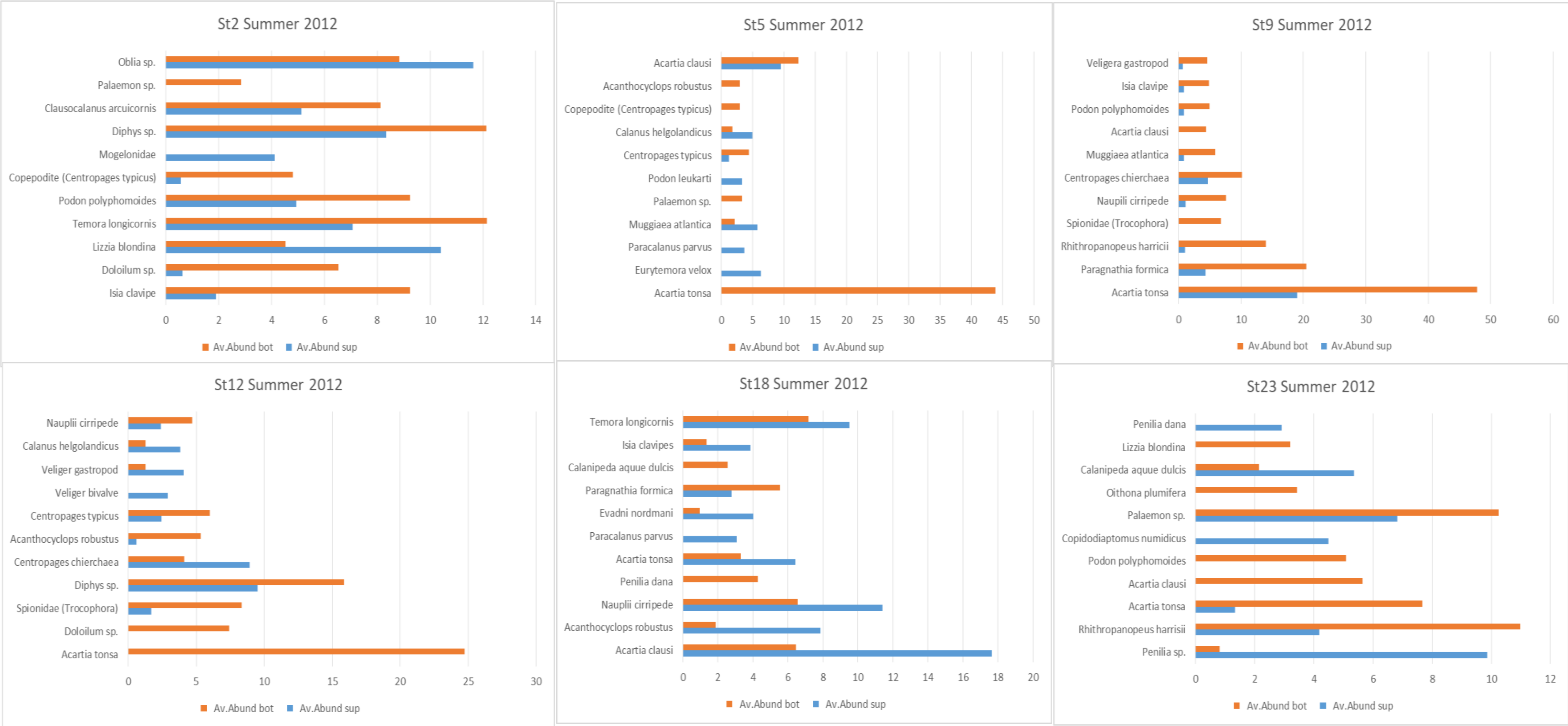

Figure 6: Average abundances for the community of zooplankton in the estuary of Mondego by station, during summer of 2012. Av. Abund bot - Average abundances at the bottom; Av. Abund sup - Average abundances at the surface. X axis with different scales.

In autumn 2012, the highest differences, between surface and bottom, were observed at stations St 12 and St 18 (Figure 7). Comparing surface and bottom samples, the station St 12 presented an increase of species on the bottom, probably due to the freshwater input associated with differences of water density (freshwater *vs* marine). At station St 18, for both surface and bottom, was registered a decrease in the number of *taxa* being *A. tonsa* the most abundant species at the surface.

The winter 2013 was marked by a great decrease of salinity in the estuary, due to the drastic increase of freshwater input, and as consequence the marine populations were flushed out of the estuary, changing the community composition (Figure 8). This effect was observed in all stations, from St 23 to St 2, by the occurrence of freshwater species, such as *Daphnia* sp., *Penilia* sp. and *Bosmina* sp. at downstream stations. Particularly, at the station St 18 there was a complete change to freshwater species on both surface and bottom samples, with a notable reduction of *taxa*.

At spring 2013 was not registered flood events as occurred in winter 2013. The marine species, with reduced presence at the estuary during flood events, were again observed during this season (Figure 9). Although, in some cases was not observed the same species that occurred before, and/or the dominant species was changed. For example, on station St 2, at this season, the dominant *taxa* was *A. clausi*, and also several species of Cnidaria such as *M. atlantica* and *Diphys* sp., that were not present on the spring 2012 (see Figure 5). At station St 5 was observed a decrease in the number of *taxa* at the bottom but an increase at the surface. At station St 9 the calanoid *Paracalanus parvus* replaced *A. tonsa* as dominant species, expressing an increase on its abundance. During this period, the estuarine community returned to the station St 18 and it was observed an increase in the abundance of *taxa* at the bottom. Indeed, the species *R. harrisii*, a true estuarine species and with a retention behavior, was observed only in the middle/upper stream estuarine stations (St 5, St 9 and St 23).

Comparing the community observed during the summer of 2012 (Figure 6) with the summer 2013 (Figure 10), the pattern of distribution of biological community changed in some stations. For example, the station St 18 showed a clear difference in abundances (see nauplius of cirripedia) and in the composition of species (for 2013, see *R. harrisii*, *Palaemon* sp., *A. robustus*). Although the change in hydrological conditions, some stations presented a similar distribution pattern.

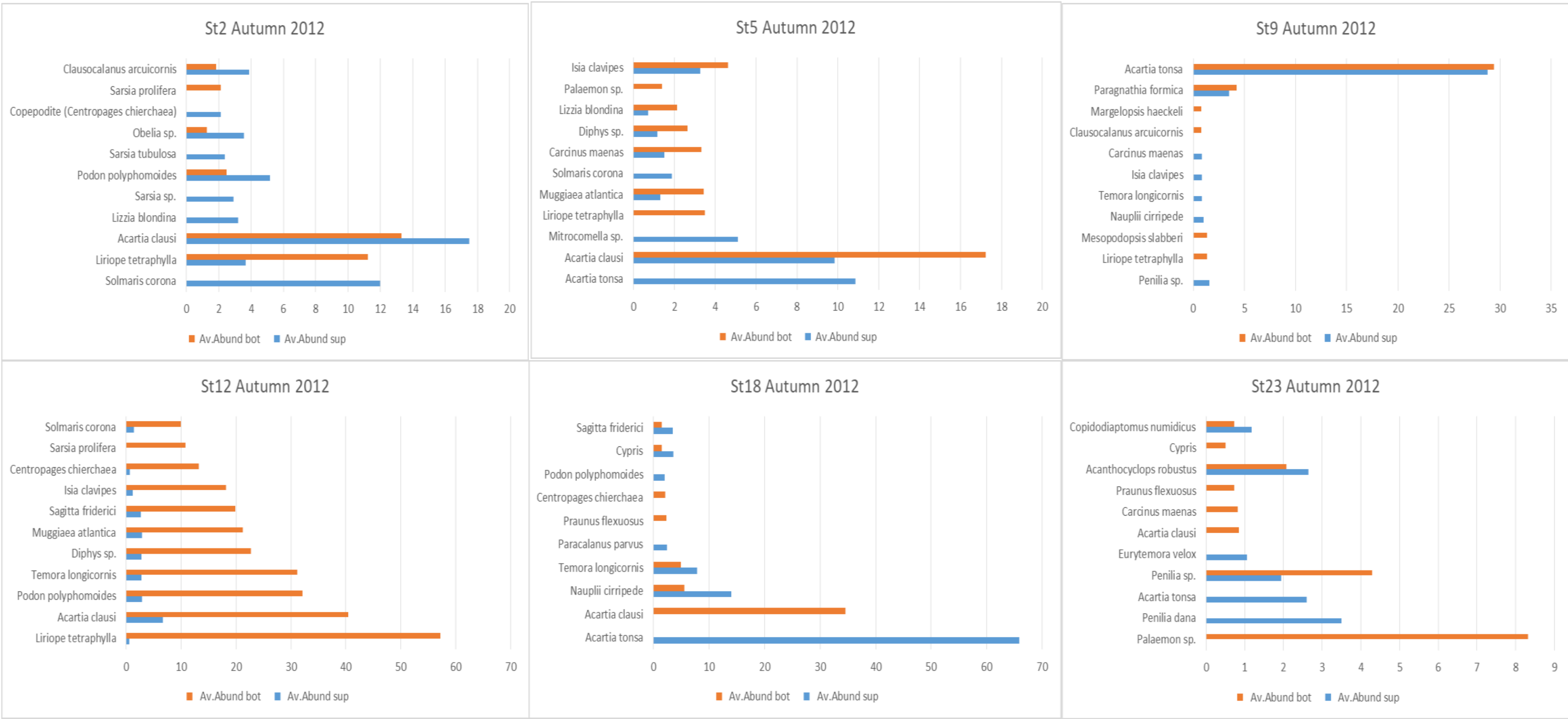

Figure 7: Average abundances for the community of zooplankton in the estuary of Mondego by station, during autumn of 2012. Av. Abund bot - Average abundances at the bottom; Av. Abund sup - Average abundances at the surface. X axis with different scales.

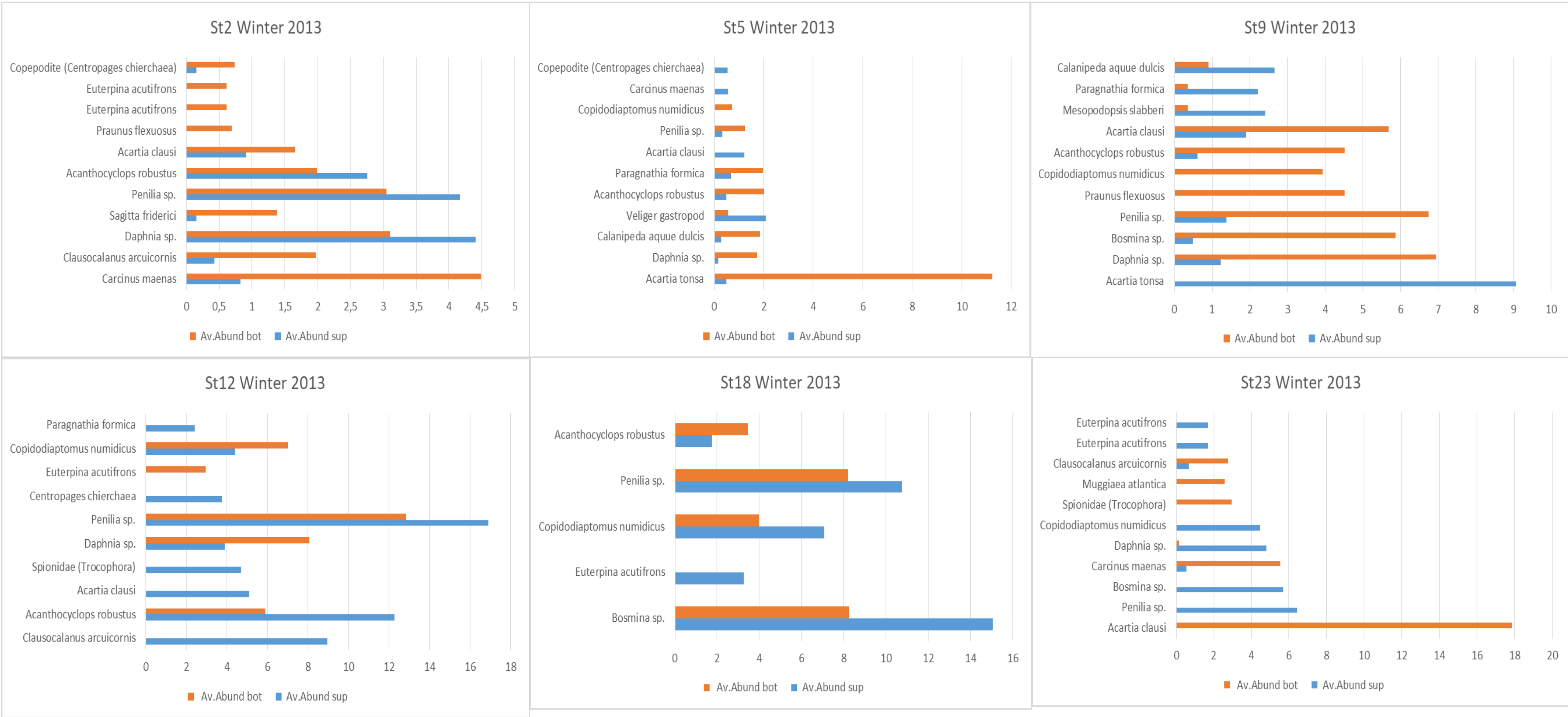

Figure 8: Average abundances for the community of zooplankton in the estuary of Mondego by station, during winter of 2013. Av. Abund bot - Average abundances at the bottom; Av. Abund sup - Average abundances at the surface. X axis with different scales.

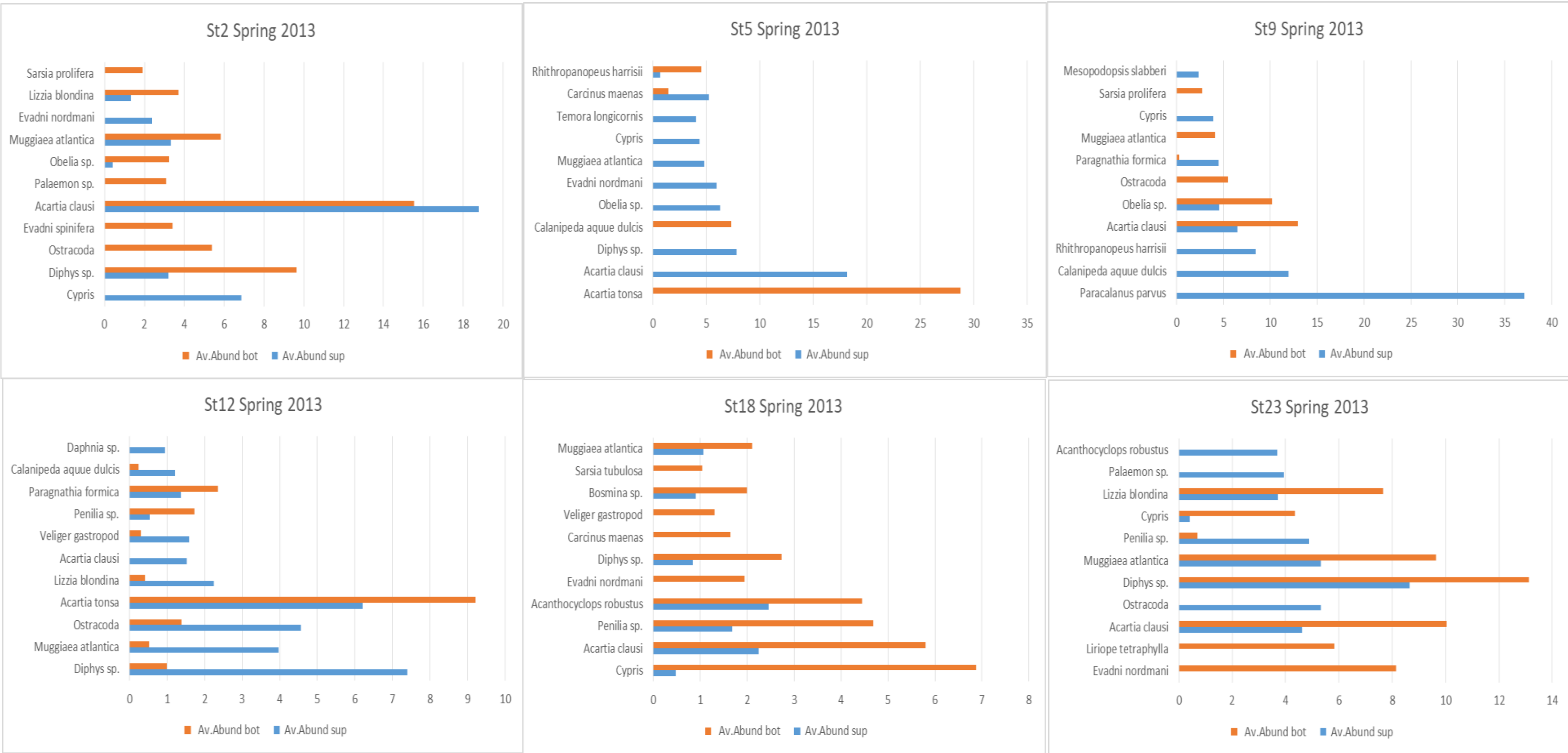

Figure 9: Average abundances for the community of zooplankton in the estuary of Mondego by station, during spring of 2013. Av. Abund bot - Average abundances at the bottom; Av. Abund sup - Average abundances at the surface. X axis with different scales.

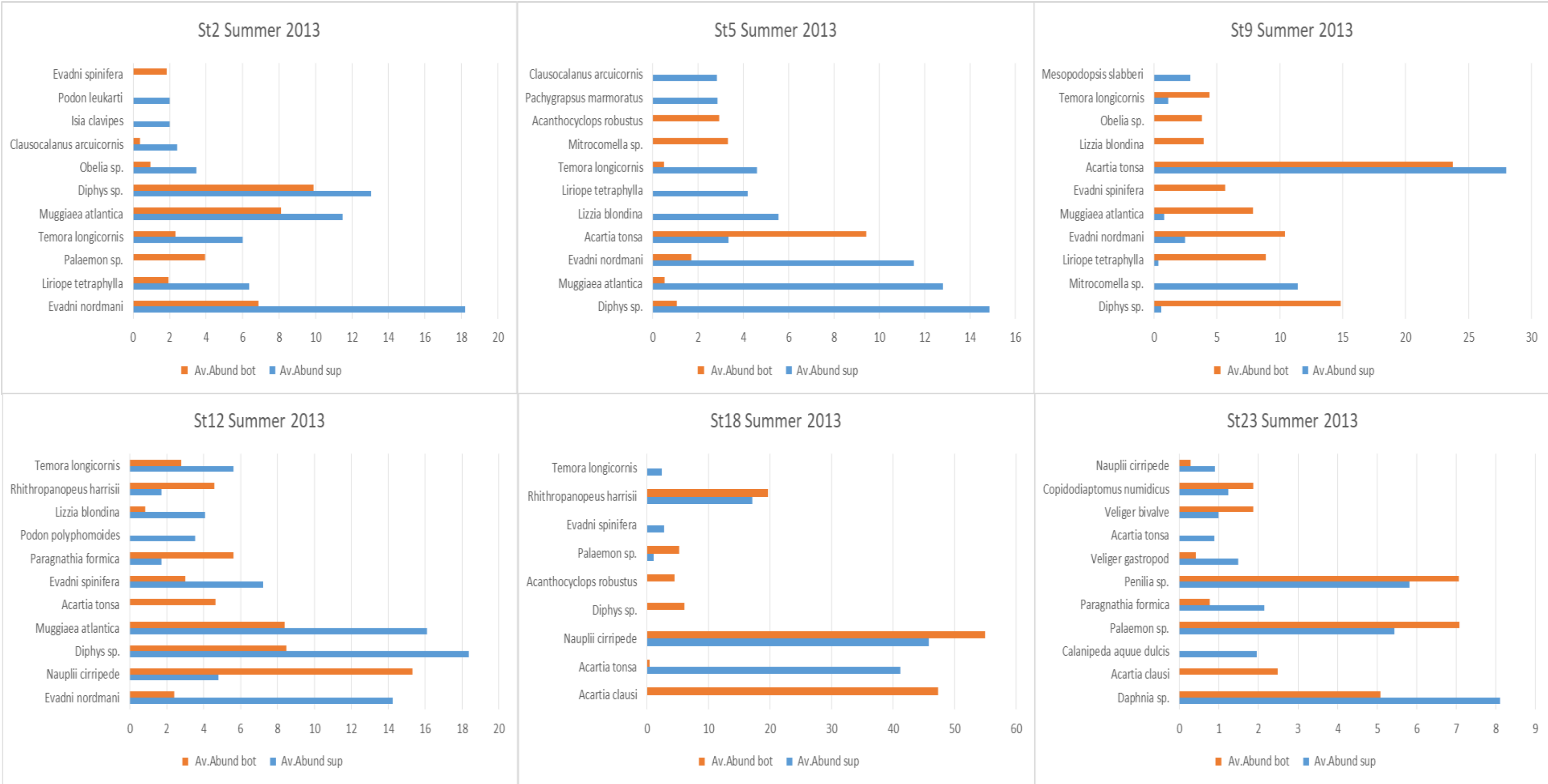

Figure 10: Average abundances for the community of zooplankton in the estuary of Mondego by station, during summer of 2013. Av. Abund bot - Average abundances at the bottom; Av. Abund sup - Average abundances at the surface. X axis with different scales.

For example, *A. tonsa* still dominated in the station St 9 (bottom and surface), probably due to their localization (south arm, near the sluice). Curiously, this species was the most abundant in the bottom at stations (St 5 and St 9) located in the south arm of estuary. The station St 2 showed a similar pattern in the *taxa* observed during the summer of 2012 and the summer of 2013. This station is located near the mouth of estuary, with a clear influence of marine water. The species *R. harrisii*, with a clear seasonal pattern (occurred during spring and summer), and an estuarine retention behavior, was observed only in stations located in the middle of estuary (St 12 and St 23, north arm), as expected.

Comparing the autumn of 2012 and 2013 the patterns of distribution of *taxa* were changed with a clear example (station St 12), where the abundance of organisms decreased (Figure 11). Overall, the dominant *taxa* changed only in two stations: St 12 and St 23. Also, in some stations, the surface community suffered a slight decrease (see St2 and St 23) on its number of *taxa*, while at the bottom level there was an increased (see St 12), suggesting that a vertical migration might occurred due to a particular changes on the environmental conditions.

As a consequence of great input of freshwater, the marine species were flushed out of the estuary (Figure 12). Thus, and like winter of 2013 (see Figure 8), all stations presented freshwater species. Comparatively to winter of 2013, it was observed a decrease of number of *taxa* and an increase of abundance at station St 9. On the contrary, at station St 18 it was observed an increase in the number of *taxa*.

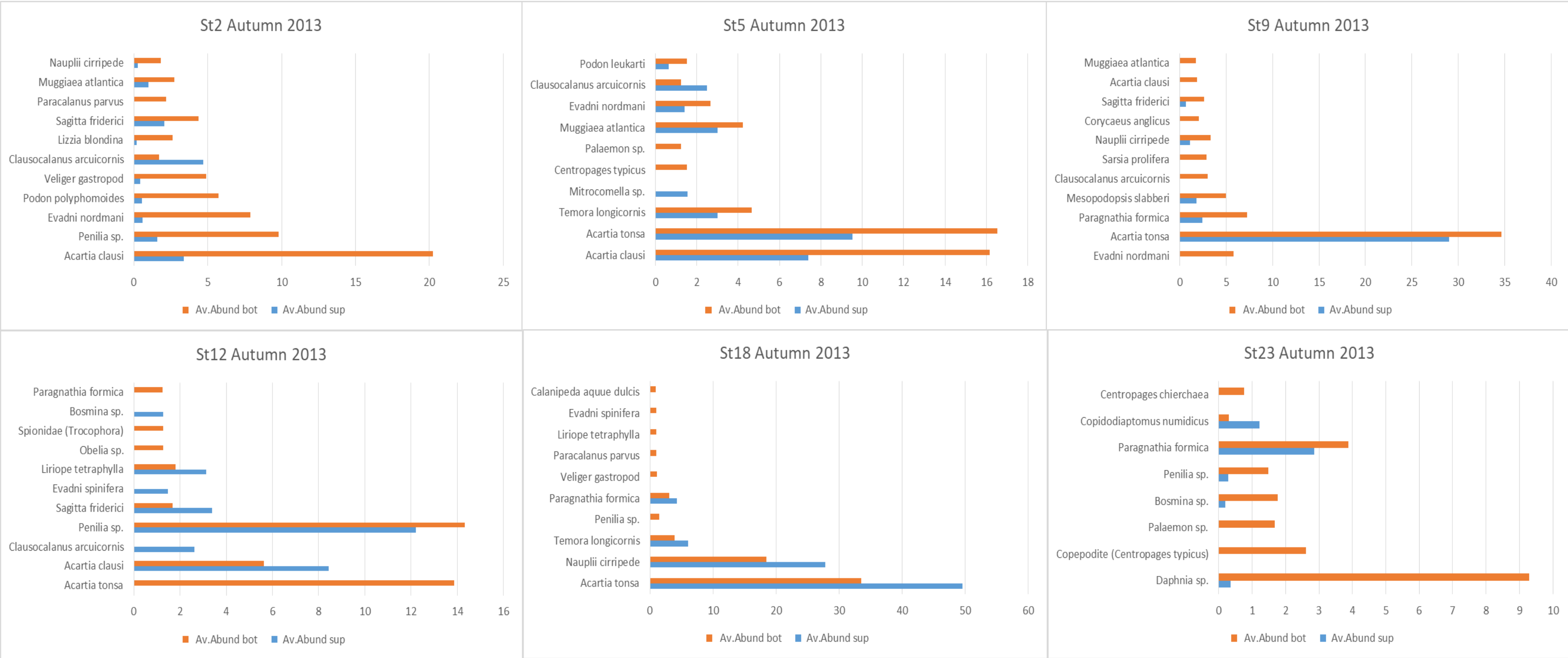

Figure 11: Average abundances for the community of zooplankton in the estuary of Mondego by station, during autumn of 2013. Av. Abund bot - Average abundances at the bottom; Av. Abund sup - Average abundances at the surface. X axis with different scales.

Figure 12: Average abundances for the community of zooplankton in the estuary of Mondego by station, during winter of 2014. Av. Abund bot - Average abundances at the bottom; Av. Abund sup - Average abundances at the surface. X axis with different scales.

4. Discussion and Conclusion

This study shows the changes on zooplankton community composition from a southern European estuarine system, in terms of diversity and abundance, according to environmental variations, mainly salinity and temperature. This work corroborate the results of previous studies in the Mondego estuary (Gonçalves *et al.*, 2010 a, b; 2012 a, b, c; Marques *et al*., 2009; Marques et al., 2017) with a shift in the plankton groups during seasonal changes. Furthermore, during extreme environmental conditions great variations main occur forcing the Zooplankton to either migrate out of the estuary (floods) or in (droughts), with the whole community expressing changes.

The middle-end of 2012 was rainy with the year of 2013 characterized by flood events, with salinity decreasing in the estuary and consequentially the marine species forced to migrate to open ocean. However the dominance of freshwater species during this period was caused by the absence of the marine species without an increase on the abundance of freshwater species. In opposite at dry period was observed an increase in marine zooplankton density and higher abundance and prevalence of marine species throughout the year (Gonçalves *et al.*, 2012; Primo et *al.*, 2009). This unusual drop of salinity also makes a huge variation in diversity during this period of time. When the salinity values come to regular values there is a slow return of the marine species which can be observed on the period from spring of 2013 to autumn 2013 after the flood event that occurred in winter 2013. Although these changes, the bottom community showed more resilience to change while the surface was more sensitive to variations. After the flood event it would be expected an increase on the zooplankton diversity (Muha et al. 2012), thought that did not happen. It was observed the species that normally occur in large numbers suffered a decrease on their abundances and some were not observed. Thus, some species that were not present start to occur on the bottom and at the surface. The drought events have a more extent effect on season that were supposed to have (Primo et al., 2009). If the flood events had occurred on dry seasons and the freshwater uptake was stable we would have similar results as the ones achieved during the drought years. But what happened was an increase of the rainfall during seasons that were supposed to making the freshwater uptake vary during the year, and on this unstable conditions the freshwater species did not have time to recover.

The marine reaches of the estuary was usually dominated by marine species, mainly the calanoid *A. clausi*, and the cyclopoid *O. nana* due to the intrusion of marine water. Copepods were clearly dominant along the estuary. Furthermore, during rainfall months, nauplii are advected from upstream areas to the mouth of the estuary. Accordingly to other works (Siokou-Frangou, 1996; Vieira et al., 2003) nauplii of copepod, copepodites and larvae of Polychaeta, Mollusca and Cirripedia showed to be important components of the plankton in the Mondego estuary. Indeed, copepods were the main dominant mesozooplankton group in the Mondego estuary, as stated on other estuarine systems (Gonçalves 2011; Kibirige and Perissinotto, 2003; Leandro et al., 2007; Uriarte and Villate, 2005).

Regarding seasonal variations, winter is characterized mainly by freshwater species and lower densities of nauplii, whereas spring and summer are dominated by marine and estuarine species, mainly juveniles. Indeed, in winter due to a higher river flow, waters are less saline and present a high concentration of nutrients from the fields. On the other hand, summer is characterized by saline and warmer water. Furthermore, the distribution and vertical patterns of copepods cannot be analyzed taking into account only the species' response to a gradient of environmental parameters (e.g. temperature and salinity). It is also needed to examine species' vertical and horizontal behaviors in terms of a dynamic complex response to tidal, diel and lunar cycles associated with environmental factors and reproductive cycles of predators and preys (Forward Jr. and Tankersley, 2001; Gonçalves 2011).

Aquatic ecosystems, mainly estuaries, reach great fluctuations which results in continuously changes and adaptations of communities to environmental factors. In an environment more and more influenced by climate changes and anthropogenic activities, severe climatic phenomena cannot be ignored, being crucial to determine the response of aquatic communities to environmental drivers and their interactions, to predict future effects of global climate change scenarios, in order to enhance monitoring and actions plans to minimize the losses in biodiversity.

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