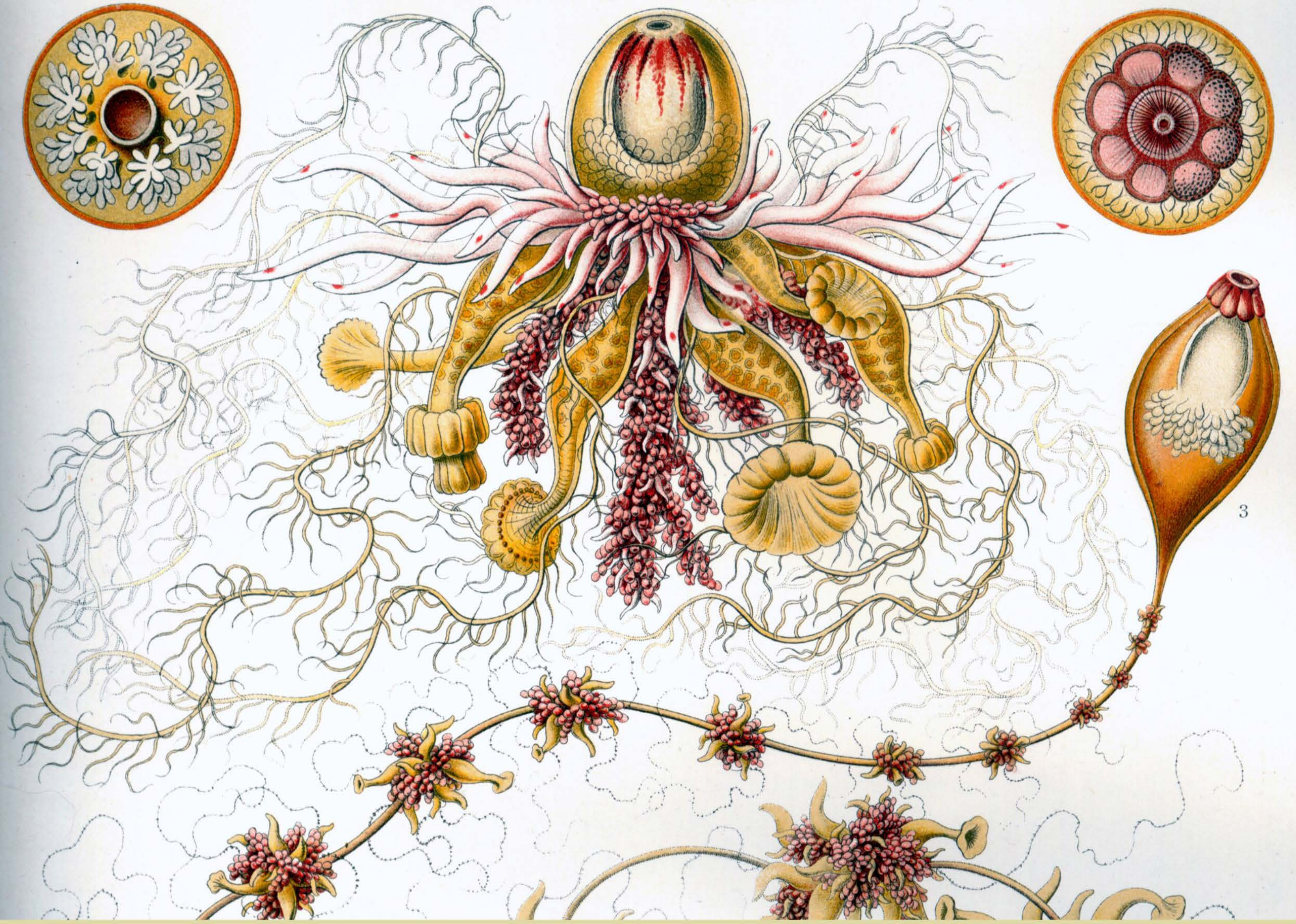

Mariaelena D'Ambrosio

Ecology of gelatinous carnivores in the Mondego estuary: the role of siphonophores

Tese de doutoramento em Biociências, ramo de especialização em Ecologia,
orientada por Professor Doutor Miguel Ângelo do Carmo Pardal, por Professor Doutor Ulisses Miranda Azeiteiro, e por Doutora Sónia Cristina Ferreira Cotrim Marques
e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra

Outubro 2017

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UNIVERSIDADE DE COIMBRA

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UNIVERSIDADE DE COIMBRA

Doctoral thesis in Biosciences, scientific area of Ecology, supervised by Prof. Dr. Miguel Ângelo do Carmo Pardal, by Prof. Dr. Ulisses Miranda Azeiteiro and by Dr. Sónia Cristina Ferreira Cotrim Marques, presented to the Department of Life Sciences of the Faculty of Sciences and Technology of the University of Coimbra

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Department of Life Sciences – University of Coimbra

Coimbra, 2017

This Thesis was supported by:

The CFE - Center for Functional Ecology, University of Coimbra and by the Portuguese Foundation for Science and Technology (FCT) through a Ph.D. grant attributed to Mariaelena D'Ambrosio (SFRH/BD/91541/2012) subsidized by the European Social Fund and MCTES (Portuguese Ministry of Science, Technology and Higher Education), through the POPH (Human Potential Operational Programme), QREN (National Strategic Reference Framework) and COMPETE (Programa Operacional Factores de Competitividade).

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Cover image: Siphonophores's illustration of Ernst Haeckel (1834-1919) from:
"Art forms in nature", 1904.

This thesis includes three manuscripts listed below, published or submitted for publication in scientific journals in the areas of ecology and marine biology:

Chapter I

D'Ambrosio, M, Molinero JC, Azeiteiro UM, Pardal MA, Primo AL, Nyitrai D, Marques SC (2016). Interannual abundance changes of gelatinous carnivore zooplankton unveil climate- driven hydrographic variations in the Iberian Peninsula, Portugal. Marine Environmental Research 120: 103–110. DOI:10.1016/j.marenvres.2016.07.012

Chapter II

D'Ambrosio M, Primo AL, Pardal MA, Martinho F, Guerrero E, Marques SC. Spatial distribution and seasonal patterns of the siphonophores *Muggiaea atlantica* and *Muggiaea kochii* in a temperate estuarine ecosystem. (Under revisions on August 2017 in Estuarine Coastal and Shelf Science).

Chapter III

D'Ambrosio M, Molinero JM, Pardal MA, Guerrero E, Martinho F, Falcão J, Azeiteiro UM, Cruz J, Marques SC.

Diel variations in the feeding behavior of *Muggiaea atlantica* in the Mondego Estuary. (Submitted on October 2017 in Journal of Plankton Research).

For Maristel and Alessio

CONTENTS

ABSTRACT

Gelatinous carnivores' zooplankton, commonly known as "jellyfish", are ubiquitous organisms in neritic systems worldwide, with an important ecological role in the pelagic food chains. During the past several decades, their ever-increasing, mostly due to environmental perturbations and climate alterations, becoming a big concern for marine ecologists, due to the implications off their blooms on the zooplankton communities.

In the Mondego estuary, the gelatinous zooplankton community is composed by higher Hydromedusae diversity and, also, by two colonial species, belonging to the order of Siphonophorae, the Calicophorans *Muggiaea atlantica* and its congener *Muggiaea kochii*, the former has constituted the most abundant gelatinous organisms in the Mondego estuary since 1994.

The first chapter aimed to describe how changes in the species richness, the phenology, and the abundance of the gelatinous carnivores of the Mondego estuary during the period 2003-2013, were influenced by the North Atlantic Oscillations and its effect on regional atmospheric variability, such as on upwelling activity. During the 11-year time series, the most abundant gelatinous species were *M. atlantica*, *Lizzia blondina*, *Obelia* sp., *Liriope tetraphylla, Solmaris corona* and *Clytia hemisphaerica*. During the period investigated, the gelatinous community displayed phenological shifts, changed their mean annual pattern from unimodal to bimodal peak, before and after 2007, respectively. Also, the species richness increased since 2007-2008, with the increase presence of rare species. Shifts on the gelatinous community were connected to the atmospheric forces promoted by the NAO and its influence on upwelling activity and regional climate, as statistical analysis confirmed.

The second chapter describes, more specifically, the spatial distribution on seasonal scale, of the two siphonophores species present in the Mondego estuary, *M. atlantica* and *M. kochii* and explained which environmental factors most influenced their life cycle in the estuary. The study was conducted during two years, 2014 and 2015, characterized by a different scenario in terms of precipitation. In fact, 2014

was considered a normal year and 2015 was dryer than the previous. Generally, in all the sampling zones, *M. atlantica* was more abundant than its congener, and both species presented higher abundance, in terms of colonies and gonophores, in the downstream area of the estuary. Moreover, during the dryer year (2015), it was observed an increase of the *M. atlantica* colonies, while the abundance of *M. kochii* was not affected by the increase in salinity values, maintaining similar abundance values during both years. Generally, salinity and prey availability have been pointed out as the determinant drivers for the reproduction and distribution of these two species in the Mondego estuary, and the positive regression coefficients denoted positive influence of salinity, calanoid nauplii, cirripedia cypris and cyclopioda copepodites on both species nectophores and gonophores.

Finally, in the third chapter, being *M. atlantica* a voracious predator in neritic waters, it was assessed the feeding activity and the selectivity of this species in the Mondego estuary on daily scale, during a half tide cycle (11 hours). For this purpose, it was analysed the gastrozooids (stomachs) content of the eudoxids (sexual stage) of *M. atlantica* estimating, also, the consumption of the different prey found in the stomachs. Generally, *M. atlantica* and eudoxids reached the higher abundance value during the flood tide and, again, the salinity was the environmental driver that most influence the presence of *M. atlantica* at daily scale, as confirmed by the multiple regression analysis and the Principal Component Analysis. Moreover, greater variability of prey was found before the high tide, corresponding, also, to the time when the largest number of full stomachs have been found. Thus, prey identified in the eudoxids stomachs (the copepod *Euterpina acutifrons* and copepod nauplii), confirmed the prey selectivity of *M. atlantica*, mostly to the small dimensions of its stomachs. It was estimated that, in the Mondego estuary, *M. atlantica* eudoxia consumed between 1.48 to 3.84 prey day^{-1}m^{-3}. These values were lower compared with others studies, probably because the night-time predation was not considered and, also, because the study analysed only the gastrozooids content of *M. atlantica* eudoxida (the sexual stage) and not of adult colonies. These findings provide baseline information to our understanding of the ecology of the gelatinous carnivorous communities in the Iberian Peninsula.

KEYWORDS

Gelatinous zooplankton; Calycophorae; *Muggiaea atlantica*; *Muggiaea kochii*; Phenological shifts; Hydrological variability; Life cycle; Feeding behavior; Temperate estuary; Iberian Peninsula

RESUMO

O zooplâncton carnívoro gelatinoso, geralmente conhecido como "água-viva" ou "jellyfish", encontra-se presente em sistemas neríticos em todo o mundo e desempenha um papel ecológico importante nas cadeias alimentares pelágicas. Durante as últimas décadas o contínuo aumento destes organismos, principalmente devido a perturbações ambientais e alterações climáticas antropogénicas, tem-se tornando uma grande preocupação para os biólogos marinhos, devido aos impactos e alterações que o seu aumento exponencial na estrutura e ecologia alimentar das comunidades marinhas.

No estuário do Mondego a comunidade zooplanctónica gelatinosa é composta em grande parte por Hydromedusae e, também, por duas espécies coloniais, pertencentes à ordem de Siphonophorae, os Calicophorans *Muggiaea atlantica* e o seu congênere *Muggiaea kochii*, sendo a primeira a espécie gelatinosa mais abundante no estuário do Mondego desde 1994.

Nesta dissertação o primeiro capítulo tem por objetivo descrever como a mudança na riqueza de espécies, a fenologia e a abundância dos carnívoros gelatinosos do estuário do Mondego, durante o período 2003-2013, foram influenciadas pela Oscilação do Atlântico Norte (NAO) e os seus efeitos na variabilidade atmosférica regional como, por exemplo, nos padrões de upwelling. Durante o estudo as espécies gelatinosas mais abundantes foram *M. atlantica, Lizzia blondina, Obelia sp., Liriope tetraphylla, Solmaris corona e Clytia hemisphaerica,* e a comunidade gelatinosa apresentou mudanças fenológicas, alterando o seu padrão anual médio, passando de um pico de abundância para dois picos, antes e depois de 2007, respetivamente. Além disso, a riqueza específica aumentou e, desde 2007-2008, as espécies raras aumentaram em abundância. Todas essas mudanças na comunidade gelatinosa estiveram ligadas as forças atmosféricas promovidas pelo NAO e a sua influência sobre os padrões de upwelling e o clima regional, como confirmou a análise estatística.

Se o primeiro capítulo teve uma abordagem em grande escala sobre toda a comunidade gelatinosa, o segundo capítulo descreve, mais especificamente, a distribuição espacial à escala sazonal das duas espécies de sifonóforos presentes no

estuário do Mondego, *M. atlantica* e *M. kochii*, explicando quais foram os fatores ambientais que mais influenciaram o seu ciclo de vida no estuário. O estudo foi realizado durante dois anos, 2014 e 2015, caracterizados por um cenário diferente em termos de precipitação. Na verdade, 2014 foi considerado um ano normal e 2015 foi mais seco do que o anterior. Geralmente, em todas as zonas de amostragem*, M. atlantica* foi mais abundante do que a sua congênere, e ambas as espécies apresentaram maior abundância, em termos de colônias e gonóforos, na área a jusante do estuário. Além disso, durante o ano de seca (2015), observou-se um aumento das colónias de *M. atlantica*, ao passo que a distribuição e a abundância de *M. kochii* não foram afetadas pelo aumento dos valores de salinidade, mantendo valores de abundância similares em ambos os anos. Geralmente, a salinidade e as presas foram apontadas como os fatores determinantes na reprodução e distribuição dessas duas espécies no estuário do Mondego e os coeficientes de regressão indicaram a influência positiva da salinidade, nauplius de calanoide, cypris de cirripedia e copepodites de ciclopoida nos nectóforos e gonóforos de ambas as espécies.

Finalmente, no terceiro capítulo, sendo *M. atlantica* um predador voraz em águas neríticas, foi avaliada a sua atividade de alimentação e a seletividade em termos de presas no estuário do Mondego numa escala diária, durante um ciclo de semi-maré. Para este propósito, foi analisado o conteúdo de gastrozóoides (estômagos) das eudoxias (fase sexual) de *M. atlantica* estimando, também, o consumo das diferentes presas encontradas nos estômagos. Geralmente, *M. atlantica* e as eudoxias atingiram o maior valor de abundância durante a enchente e, novamente, a salinidade foi a variável ambiental que mais influenciou a presença e a variabilidade diária de *M. atlantica*, resultado confirmado pela análise de regressão múltipla e da Análise de Componentes Principais. Além disso, maior variabilidade de presas foi encontrada antes da maré alta, que também correspondeu ao momento em que o maior número de estômagos cheios foram encontrados. Assim, as presas identificadas nos estômagos das eudoxias (o copepode *Euterpina acutifrons* e náuplios de copepoda), confirmaram a seletividade em termos de presas devido, principalmente, às pequenas dimensões dos seus estômagos. Estima-se que, no estuário do Mondego, as eudoxias de *M. atlantica* consumiram

entre 1,48 a 3,84 presas^{-1}m^{-3} d^{-1}, resultados baixos em comparação com outros estudos, provavelmente porque a predação noturna não foi considerada e, também, porque o estudo analisou apenas o conteúdo de gastrozóoides das eudoxias de *M. atlantica* (o estágio sexual) e não o de colónias adultas.

PALAVRAS-CHAVES

Zooplâncton gelatinoso; Calycophorae; *Muggiaea atlantica; Muggiaea kochii*; Mudanças fenológicas; Variabilidade hidrológica; Ciclo de vida; Comportamento alimentar; Estúario temperado; Península Ibérica

CHAPTER 1

GENERAL INTRODUCTION

1.1 Gelatinous carnivore zooplankton

“Jellyfish” is a generic term referring to free-floating gelatinous carnivores zooplankton belonging to the Phylum Cnidaria, which includes the Classes Scyphozoa, Hydrozoa and Cubozoa (Graham et al., 2014; Richardson et al., 2009) (Fig. 1.1). This term included also the gelatinous species belonging to the Phylum Ctenophora (comb jellies) and Chordata (larvaceans, salps, doliolids, and pyrosomes) (Boero et al., 2008; Graham et al., 2014).

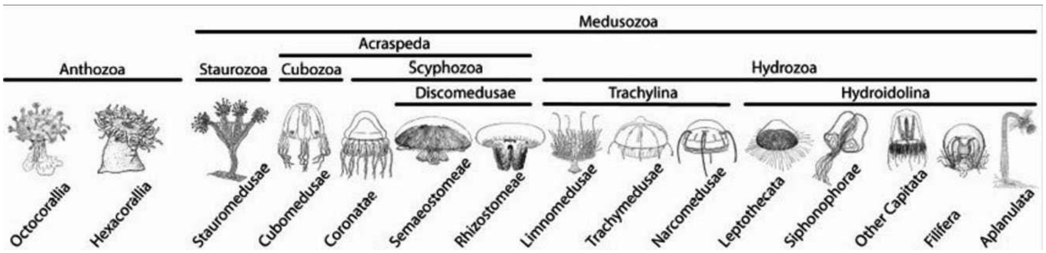

Figure 1.1: Classification of the phylum Cnidaria (Collins et al., 2003).

They are considered as crucial organisms for the ecosystems, because they can affect nekton in the pelagic web, both by competition (by feeding on the potential prey of fish: the crustaceans), by direct predation on nekton (feeding on fish eggs and larvae) (Bouillon et al., 2006; Purcell, 1992; Sabatés et al., 2010) and by linking primary production to higher trophic levels and deep-sea communities (Condon et al., 2012) (Fig. 1.2).

Gelatinous carnivores, predominantly marine species, may have complex life cycle, which varies with their taxonomy (Daly et al., 2007). Under suitable environmental conditions, they have the ability to reproduce rapidly, by asexual reproduction, forming blooms, that cause negative ecological and socio-economic impacts. Indeed, their blooms can reduce commercially fish stocks (Lucas et al., 2014), can limit bioavailable carbon to higher trophic levels, (Condon et al., 2011), causing detrimental economic impacts on aquaculture, tourism and coastal infrastructure (Purcell et al., 2007).

The gelatinous component of plankton ‘mysteriously’ appears and disappears at unpredictable times, and is usually considered “as pertaining to the dark side of ecology” (Boero et al., 2008). Over recent decades, several cases of outbursts of indigenous jellyfish have been documented and/or reviewed (Brodeur et al., 2002; Mills, 2001; Purcell, 2005), as well as evidences of relationships between gelatinous zooplankton mass occurrence and anthropogenic perturbations, such as global warming and eutrophication (Brodeur et al., 2008; Purcell et al., 2007; Richardson, 2008). Moreover, in many estuarine and coastal ecosystems, where the stability of the water column promotes the increase and aggregation of gelatinous carnivores, high numbers of gelatinous zooplankton species have been reported (Graham et al., 2001; Licandro et al., 2012). In coastal areas, eutrophication is the major reason to develop small organisms´ blooms related with decomposing on dead phytoplankton resulting in accelerate consumption of the oxygen in the water column. High tolerance of gelatinous organisms in low oxygen concentrations, can endure with benefits from the exclusion of potential competitors (Purcell et al., 2015, 2007; Richardson et al., 2009).

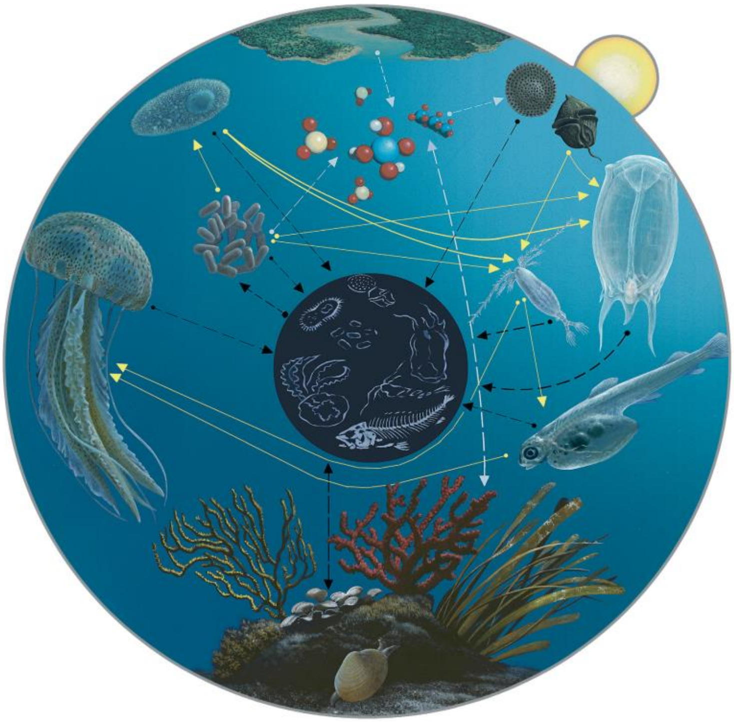

Figure 1.2: Schematic representation of the trophic fluxes in the water column. White arrows: biogeochemical fluxes; black arrows: fluxes of particulate organic matter. Yellows arrows represent trophic links (Boero et al., 2008).

Climatic variability has well documented effects on gelatinous zooplankton, including abundance, productivity, geographical distribution (Richardson et al., 2009) and phenology (Blackett, 2015). Moreover, these organisms are poikilothermic, therefore not able to regulate internally environmental temperature (Richardson, 2008). Hence, temperature changes in the marine environment directly affect their fundamental functional processes, such as ingestion, respiration and reproductive development (Richardson, 2008).

The marine environment is changing rapidly (Philippart et al., 2011). Figure 1.3 shows how average sea surface temperature around the world changed since 1880 until now. It is clear an increasing during the 20th century that continues nowadays (Levitus et al., 2000). From 1901 through 2015 (Fig. 1.4), temperature rose at an average rate of 0.07°C per decade, being consistently higher during the past three decades than at any other time since reliable observations began in 1880 (NOAA, 2016a) (Fig. 1.3). Based on the historical record, increases in sea surface temperature have largely occurred over two key periods: between 1910 and 1940, and from about 1970 to the present. Sea surface temperature appears to have cooled between 1880 and 1910 (Fig. 1.3). Moreover, during the last 10-15 years, sea water temperature throughout much of the globe have changed at unprecedented rates (Philippart et al., 2011). These changes suggested increasing perturbations in ocean conditions, that might have benefited gelatinous plankton biomass pulses (Attrill et al., 2007; Boero et al., 2008).

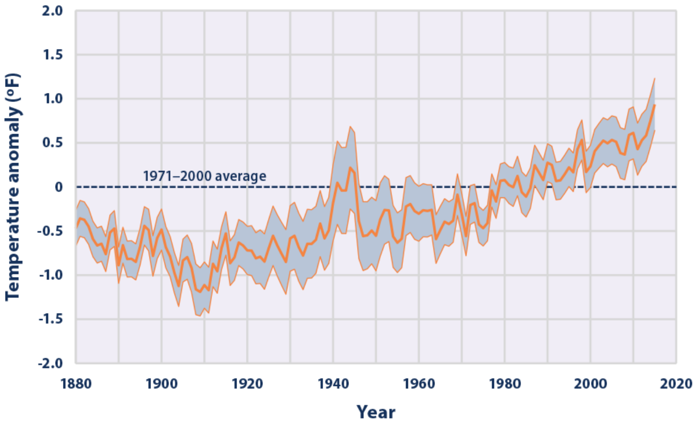

Figure 1.3: Average Global Sea Surface Temperature, 1880–2015. This graph shows how the average surface temperature of the world's oceans has changed since 1880. This graph uses the 1971 to 2000 average as a baseline for depicting change (NOAA, 2016a).

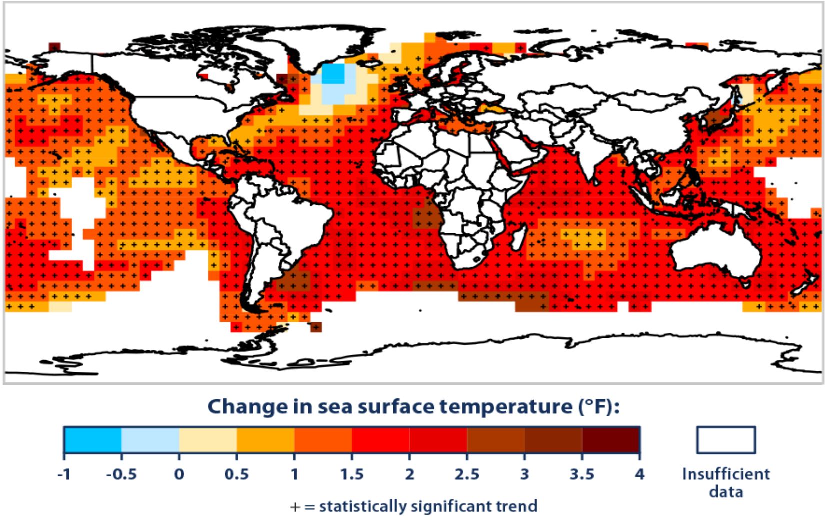

Figure 1.4**:** Change in Sea Surface Temperature, 1901–2015 (NOAA, 2016b).

1.2. Siphonophores and calicophorans *Muggiaea Atlantica* (Cunningham, 1892) and *Muggiaea Kochii* (Will, 1844)

Siphonophores are pelagic, free- swimming or floating Hydrozoa, belonging to the phylum Cnidaria, constituted by three suborders: Cystonectae, Physonectae and Calycophorae (Bouillon et al., 2006; Daly et al., 2007; Mapstone, 2014). They are very abundant and one of the major carnivorous groups of the plankton (Carré and Carré, 1991). In the adult phases they form polymorphic colonies, highly specialized and complex (Bouillon et al., 2004; Dunn, 2009), with a length that varies from millimeters to several meters (Carré and Carré, 1991; Silguero and Robinson, 2000), including the longest animal in the world (>40 meters) (Dunn, 2005; Robison, 1995). Siphonophores are composed by "zooids", units genetically identical, but structurally different and functionally specialized (Carré and Carré, 1991; Dunn 2005), attached to a central stolon, supported by a floating and swimming system (Bouillon et al., 2004). Throughout the life of the colony, zooids remain attached and integrated to one another, having highest division of labor, and acting as unitary multicellular animals (Dunn, 2009; Mackie et al., 1987). Moreover, zooids associated with the gonophores in repetitive groups along the stolon, forming the cormidia (cormidium, as singular) (Fig. 1.5), creating the siphosome of the colony, whereas floating system and nectophores forming the nectosome (Fig. 1.5). The adult colony is, also, referred to as polygastric stage (Bouillon et al., 2004).

Regarding feeding, siphonophores are ambush carnivores predators (Batistić et al., 2013; Purcell, 1981; Purcell and Arai, 2001), feeding mostly on copepods and fish larvae, but also other zooplankton organisms, such ostracods, chaetognaths (Licandro et al., 2012; Purcell, 1983) and sometimes on decapod larvae (Mapstone, 2014). The three suborders have different diet, depending by the dimension of their gastrozooids (Purcell, 1983).

Despite their abundance, the knowledge about siphonophores is still poor because the colonies are very fragile and, most of time, they are defeated in pieces by the nets, and only parts of them are found in the samples (Carré and Carré, 1991; Kirkpatrick and Pugh, 1984; Purcell, 1981).

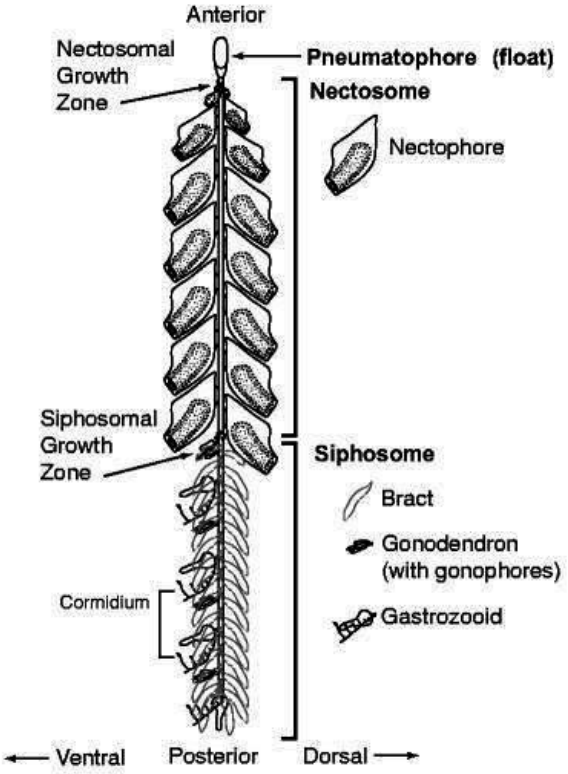

Figure 1.5: Diagrams of siphonophore´s colony structure (Dunn et al., 2005).

1.2.1 Structure of the colony

All the information contained in the next paragraph is based on Bouillon et al., (2004) and Mapstone et al., (2014).

Pneumatophore: it is a cavity divided in chambers in the front part of the colony, the pneumatophore, or epical float, is a characteristic only of the suborders of Cystonectae and Physonectae (Fig. 1.5).

Nectophore: the nectophores, or swimming bells, are muscular structures widely adapted to swim. As meduase, they have an umbrella, subumbrellar cavity and radial canals, but do not have manubrium, mouth or tentacles (Fig. 1.5).

Pneumatophore and nectophores formed the nectosome of the colony.

Bracts: generally, bracts have a protective, floating and sensory function and, in the species without nectophores, they, also, have developed swimming role (Fig. 1.5).

Gonodendron and Gonozooids: Gonodendron is constituted by several gonozooids (or gonophores) attached to the stem and can be monoecious (with both male and female gonophores on the same individual), or dioecious (species with separate sexes) (Mapstone, 2014). Physonectae female gonophores produce only one egg, but their cormidia generate a succession of several male and female gonophores. In the suborder of calycophorae, gonophores produce several eggs (2 to 30), and their cormidia are liberated in the end of the stolon as eudoxid (the sexual stage), a free-swimming larval stage composed by a bract, a gonophore and capable to feed itself because equipped with one stomach. During its free- swimming larval stage, bract of the eudoxia can generate and relieve several male and female gonophores alternated (Mapstone, 2014) (Fig. 1.5)

Gastrozooids: responsible for feeding the colony, they are the only zooids able of ingesting food (Fig. 1.5)

Bracts, gonozooids and gastrozooids form the siphonome, the posterior part of the colony (Fig. 1.5).

1.2.2 Life cycle

The siphonophores life cycle is similar to that of hydroids and it is illustrated in the Figure 1.6. The gonophores release the egg or the sperm and, after fertilization, the planula in the hydroids forms a larval hydroid that attaches to the substrate and grows, developing into the adult colony. In the siphonophores life cycle, the planula remains pelagic and, in ca. 24 hours, gives rise to specialized pelagic larvae, siphonula in the order of Physocetae (Fig 1.6a) or calyconula in the order of Calycophorae (Fig. 6b), developing directly into the polygastric stage (Bouillon et al., 2006; Mackie et al., 1987) (Fig. 1.6). In the latter order, from the calyconula larvae develops a young colony, and its nectophores can be replaced by one (anterior) or two (anterior and posterior) nectophores (Mackie et al., 1987).

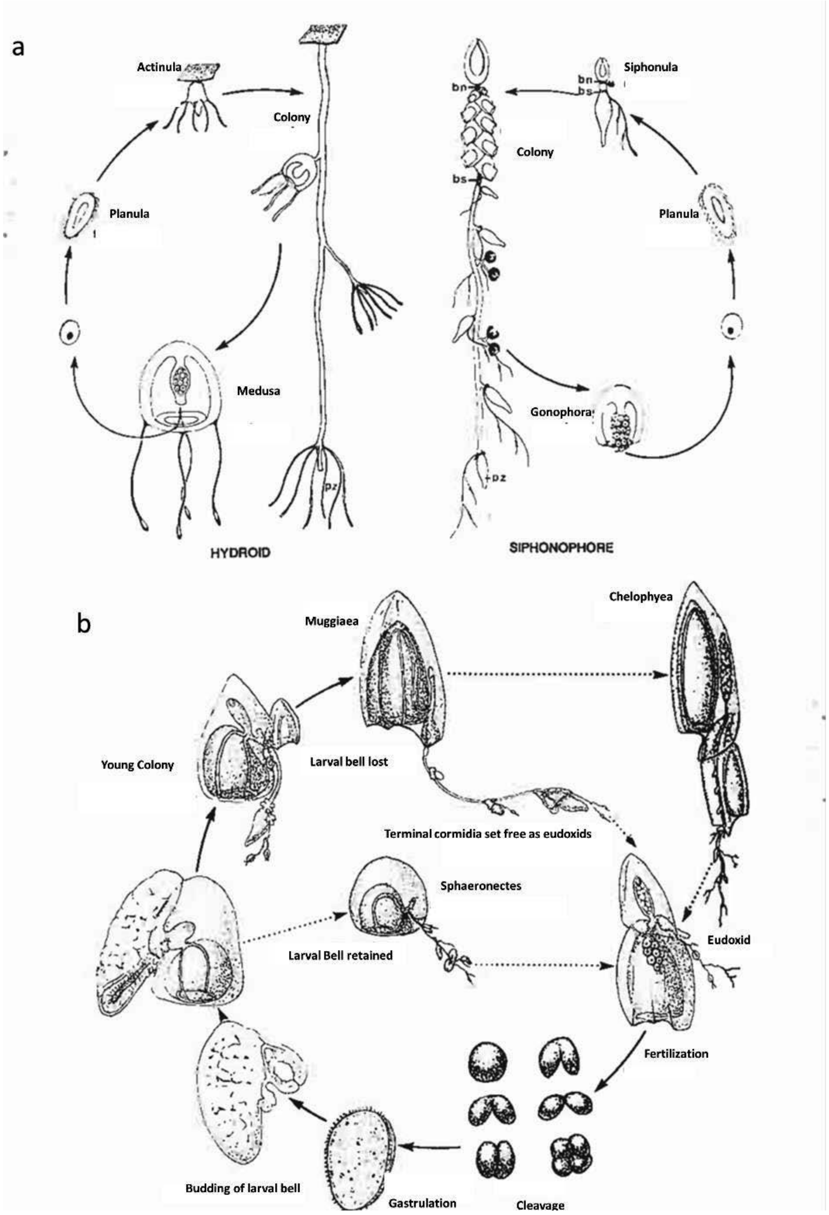

Figure 1.6: Comparison between life cycle of Hydroids, (a) Physocetae and (b) Calycophorae (Mackie et al., 1987).

Muggiaea atlantica and *Muggiaea kochii* are two siphonophores species belonging to the suborder Calycophorae (Mackie et al., 1987). These two species are very abundant components in neritic waters of temperate regions (Batistić et al., 2013), mostly during spring-summer period (Angeles, 1971; Gili et al., 1987), representing the order of siphonophores in the study area of this dissertation. About their distribution, *M. atlantica* is a typical Atlantic species, where is a very abundant component of neritic gelatinous zooplankton communities (Mapstone, 2014). Otherwise, its congener *M. kochii* prefers higher water temperature, thought is present in several Atlantic neritic systems (Mapstone, 2014).

The particularity of these two species is, despite most calycophorans have an anterior and posterior nectophores, *Muggiaea* species develop only one anterior nectophore, which is very similar between the two species (Mapstone, 2014) (Fig. 1.7). The most obvious difference between them, is the length of the hydroecium, a furrow developed in the nectophores (Bouillon et al., 2006) which is shallower in *M. kochii* than in *M. atlantica* (Fig. 1.8a, 1.8c). Nevertheless, the eudoxids released from the colony are morphologically indistinguishable between the two species (Fig. 1.8d) (Kirkpatrick and Pugh, 1984).

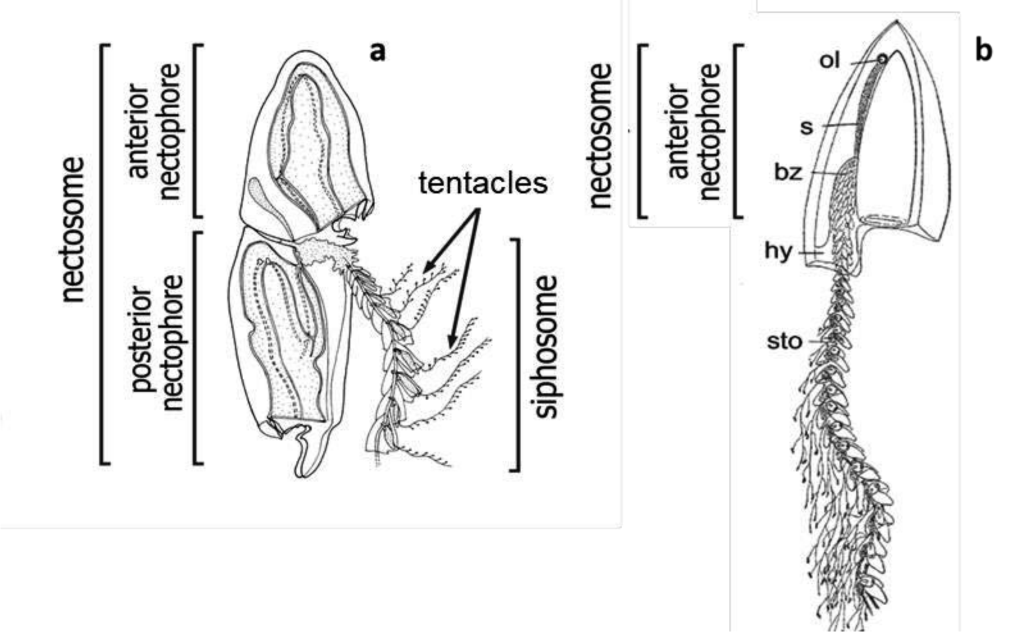

Figure 1.7: Example of a calycophoran *M. atlantica* with different element of the colony. ol: oleocyte; s: somatocys; bz: budding zone; hy: hydroecium; sto: stolon (Bouillon 2006).

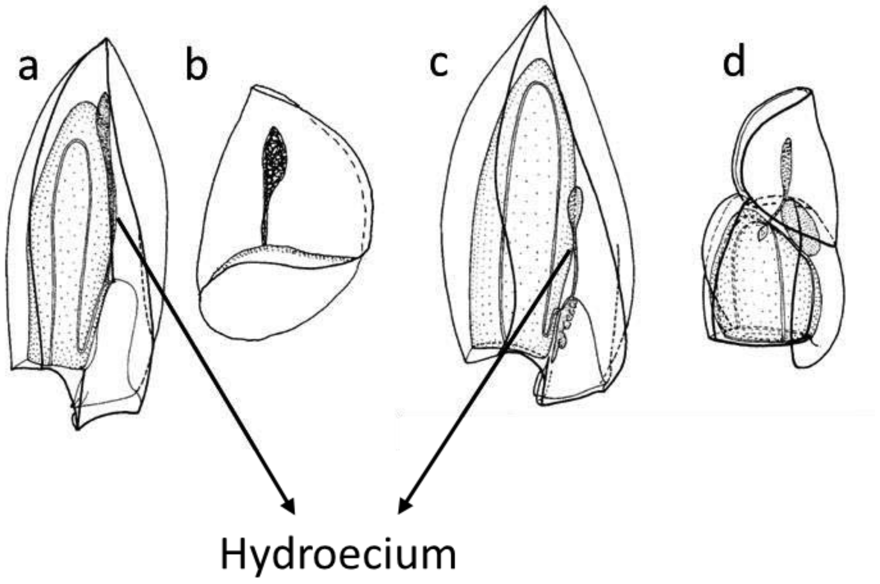

Figure 1.8: a: *M. atlantica* nectophore; b: *M. atlantica* bract; c: *M. kochii* nectophore; d: *Muggiaea* spp. eudoxid (Bouillon et al., 2006)

1.3 The importance of the estuarine systems

Estuaries are considered decisive ecosystems, being inhabited by a large range of invertebrates. Estuaries are feeding grounds for a lot birds' species (Wołowicz et al., 2007) and provide nursery areas for numerous species of marine fish, which are dependent on this system for the early stage of their growth (Lamberth and Turpie, 2012). These ecosystems represent the great transition between freshwater and marine biomes, and as such are influenced by both aquatic realms (Wołowicz et al., 2007). They are unique places, strongly affected by tidal action, where river and sea merge into a dynamic natural complex (Liu and Liu, 2014). The physical and chemical dynamics and the ecology of estuarine areas are strongly influenced by the freshwater runoff and the adjacent open sea (Duarte et al., 2001). The freshwater input influences estuarine hydrology by creating salinity gradients and stratification and assures large transport of silt, organic material and inorganic nutrients to the estuaries. Otherwise, the open marine areas determine physical and chemical forcing on the estuarine ecosystem (Duarte et al., 2001).

The dynamics of estuarine communities are influenced by the interactions of both physical and biological processes (Kimmerer, 2004). It is important to highlight that, at shorter time scale, physical effect, e.g. variation in freshwater flow, tidal moving and salinity distribution, tend to dominate (Kimmerer, 2004).

In the estuarine systems on the Northern hemisphere, one of the main force that influences the river flow regime and water circulation is the North Atlantic Oscillation (NAO) (Trigo et al., 2004). NAO is an atmospheric circulation pattern with no particular periodicity that is depending on the difference of atmospheric pressure at the sea levels of two stations across the North Atlantic Ocean between the Iceland and over the Azores, which mainly control the westerlies toward Europe (Hurrell, 1995) (Fig. 1.9).

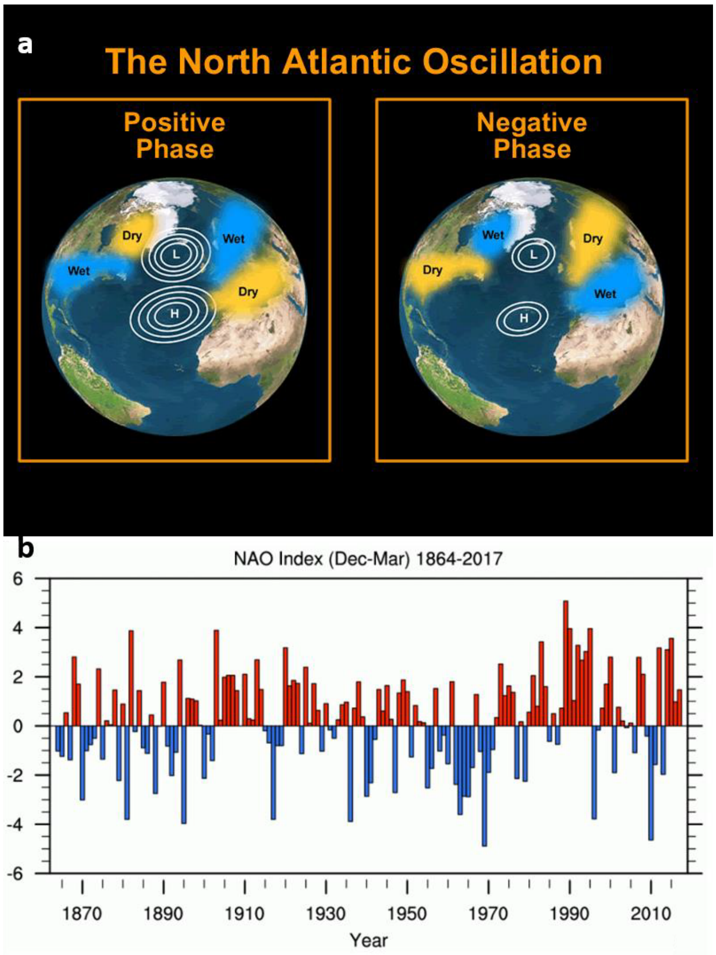

Figure 1.9: The North Atlantic Oscillation circulation. a: NAO's positive and negative phases and their influence on the North Atlantic hemisphere climate (Source: University Corporation for Atmospheric Research https://www2.ucar.edu/). b: Winter (December through March) index of the NAO, based on the difference of normalized sea level pressure (SLP) between Portugal and Iceland, since 1864 to 2017 (Hurrell and Research, 2017).

Its relative strengths, positions and direction vary from year to year, affecting wind speed, temperature, moisture distribution, intensity, the number and track of storms (Hurrell et al., 2003). Strongly positive NAO (NAO+), creates a large pressure gradient across the North Atlantic driving winter storms across the Atlantic and into Northern Europe (Fig. 1.9a). In the western coast of the Iberian Peninsula, one of the four major upwelling regions in the world (Santos et al., 2011 and references therein), positive changes of the NAO enhanced the variance in regional wind and precipitation, increasing the upwelling activity, promoting nutrient-rich water toward the ocean (Lynam et al., 2004; Santos et al., 2011). In contrast, during the negative phase, there is only a small pressure gradient. Southern Europe and Africa receive weak winter storms while Northern Europe and the eastern United States are cold and dry (Fig. 1.9b) (Hurrell and Research, 2017).

Estuarine ecosystems are highly sensitive to both climate changes and the mounting anthropogenic disturbances which, by altering coastal habitats, magnify the effects of climate (Marques et al., 2007). The increase of the human activities close to the coastal waters and estuaries and the consequent reduction in river flow contributed for decreasing the water quality (Philippart et al., 2011; Rocha et al., 2002). Thus, the synergies of both climate and anthropogenic pressures modify biotic and abiotic environmental settings, and may ultimately shift hydrographic dynamics, structure, and function of aquatic communities, causing sever changes in these ecosystems (Chícharo et al., 2009; Marques et al., 2014). Moreover, in recent years, extensive outbreaks of gelatinous zooplankton have been recorded in several estuarine and coastal ecosystems, raising concern about substantial changes in the entire pelagic ecosystem dynamic (Condon et al., 2012; Lucas et al., 2014). Particularly in estuaries, salinity significantly affects population size and asexual reproduction of cnidarians (Purcell, 2005). In fact, it affects gelatinous asexual reproduction rates directly through metabolism, and indirectly through prey capture (Purcell, 2005).

1.4 The Mondego estuary as case study

All the studies presented in this thesis were carried out through surveys performed in the Mondego estuary (40º08’N, 8º50’W), a polyhaline and mesotidal system located in in a temperate region on the west cost of Portugal (Fig. 1.10). This system is approximately 25 Km long, occupying a total area of 8.6 Km², with an average depth of 6-7 meters and tidal amplitudes ranging from 3 to 6 meters (Duarte et al., 2002). This estuarine system is divided into two arms (north and south), separated by a large sediment island, the Murraceira Island, and characterized by different hydrological characteristics. The north arm is 21 km long, with an area of 5.87 Km², is deeper (4–8 m at high tide), the residence time is of 2 days (Azeiteiro et al., 1999)and receives the majority of freshwater input from Mondego River. At neap tides, this arm is characterized by a salt-wedge during low tide, changing to partially mixed water column at high tide (Cunha and Dinis, 2002). Contrasting situations occur at spring tides, when it is partially mixed at low tide and well mixed at high tide (Cunha and Dinis, 2002). The southern arm is 7 km long, with an area of 2.71 km², is shallower (2–4m deep, at high tide), with higher residence times (2–8 days), characterized by large areas of exposed intertidal flats during low tide, with several seagrass meadows and saltmarshes at the downstream area. The water circulation in the south arm is predominantly due to the tidal cycle and to a relatively small freshwater input from a tributary located about 6 km from the mouth, the Pranto River, which is artificially controlled by a sluice (Marques et al., 2006). Over the period 1990-1998, the south arm was under severe environmental stress due to human activities, namely an ongoing eutrophication process, industries, aquaculture farms and nutrients discharge from agricultural lands located on the lower river Mondego valley. After 1998, this river arm has been gradually restored do to an awareness program (Cardoso et al., 2010; Dolbeth et al., 2011; Grilo et al., 2011).

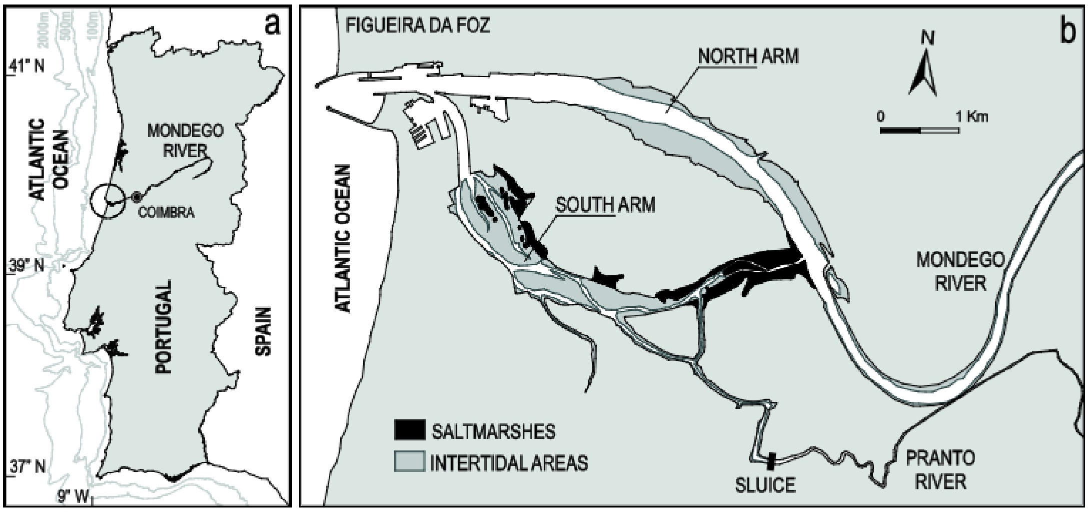

Figure 1.10: (a) Location of the Mondego estuary on the west coast of Portugal; (b) detailed scheme of the estuary showing the two arms and the intertidal and saltmarsh areas.

In the Mondego estuary, tidal movement is one of the major driving forces of estuarine circulation in this system, characterized by a semi-diurnal cycle, with a median daily tidal range of 2.5 meters, and a full tidal period covering about 12.4 hours (Duarte et al., 2002). Moreover, most of the estuarine processes (physical, chemical and biological) are related to salinity, because its variation is one of the major characteristics of an estuarine system (Duarte et al., 2001). The upper area of the Mondego estuary is considered the freshwater section, still with a tidal influence, but with salinity close to zero during most of the year. The middle section, is the salinity-mixing zone, whereas in the lower area, salinity is usually very close to sea water values.

1.5 Thesis aims and general objectives

Previously to the present dissertation, the zooplankton community in the Mondego estuary was thoroughly explored. Indeed, since 2003, spatial and temporal patterns of the zooplankton community were investigated, identifying the main influent environmental factors that most influenced it (Primo et al., 2015). Moreover, the role of seasonal, lunar and diel cycles on zooplankton community were studied (Marques et al., 2009) and, also, how these communities responded to the climate variability (e.g. severe droughts and consequently lower precipitation regime) (Marques et al., 2007; Primo et al., 2011). In addition, Primo et al. (2012) examined the impact of large-scale climatic variables on gelatinous carnivore zooplankton, and showed how water temperature mostly influenced the community and its blooms in the Mondego estuary. In all those several studies, *M. atlantica* always represented the most abundant species in terms of gelatinous carnivores.

Thus, given the remarkably importance of gelatinous carnivores zooplankton in the pelagic food chains (West et al., 2009), the present dissertation aimed to explain which were the environmental factors that mainly influenced their distribution and abundance in the Mondego estuary focusing, particularly, on the life cycle, abundance and feeding behavior of *M. atlantica*, the most abundant gelatinous carnivore species, and its congener *M. kochii* in three different temporal scales (long-term, seasonal and daily scale).

To achieve such objectives, field studies were conducted and conceptually divided into three chapters.

Using data time series of gelatinous carnivores community collected during a decade as a part of a long-term zooplankton research that began in 2003 (Marques et al., 2014), the first chapter aimed to investigate the potential influence of large-scale atmospheric phenomena, as North Atlantic Oscillation (NAO), on regional Mondego estuary climate, tried to explain how such cascading effect influenced species richness and phenology of the gelatinous carnivore community. In the second chapter (seasonal scale) is described the influence of environmental factors, such as temperature, salinity and prey availability on the seasonal abundance patterns on the spatial distribution of *M. atlantica* and *M. kochii* in Mondego estuary (years 2014 and 2015). (considering prey caught with 200 µm mesh-size net). Finally, third chapter aimed to describe the trophic links and investigate dietary behaviour of *M. atlantica* species over a half-tidal cycle, quantifying its predation impact and prey selectivity on a daily scale, through the analysis of the *M. atlantica* eudoxids stomach content. Thus, were considered prey caught with 335 and 64 µm mesh-size nets and the amount of carbon and nitrogen assimilated from the eudoxids were estimated.

CHAPTER 2

INTERANNUAL ABUNDANCE CHANGES OF GELATINOUS CARNIVORE ZOOPLANKTON UNVEIL CLIMATE-DRIVEN HYDROGRAPHIC VARIATIONS IN THE IBERIAN PENINSULA, PORTUGAL

2.1 Abstract

The persistent massive blooms of gelatinous zooplankton recorded during recent decades may be indicative of marine ecosystem changes. In this study, we investigated the potential influence of the North Atlantic climate (NAO) variability on decadal abundance changes of gelatinous carnivore zooplankton in the Mondego estuary, Portugal, over the period 2003-2013. During the 11-year study, the community of gelatinous carnivores encompassed a larger diversity of hydromedusae than siphonophores; the former dominated by *Obelia* spp., *Lizzia blondina, Clythia hemisphaerica, Liriope tetraphylla and Solmaris corona*, while the latter dominated by *Muggiaea atlantica*. Gelatinous carnivore zooplankton displayed marked interannual variability and mounting species richness over the period examined. Their pattern of abundance shifted towards larger abundances ca. 2007 and significant phenological changes. The latter included a shift in the mean annual pattern (from unimodal to bimodal peak, prior and after 2007 respectively) and an earlier timing of the first annual peak concurrent with enhanced temperatures. These changes were concurrent with the climate-driven environmental variability mainly controlled by the NAO, which displayed larger variance after 2007 along with an enhanced upwelling activity. Structural equation modelling allowed depicting cascading effects derived from the NAO influence on regional climate and upwelling variability further shaping water temperature. Such cascading effect percolated the structure and dynamics of the community of gelatinous carnivore zooplankton in the Mondego estuary.

2.2 Keywords

Gelatinous carnivore zooplankton, North Atlantic Climate, NAO, Upwelling, Mondego estuary.

2.3 **Introduction**

Marine ecosystems are sculpted by both the anthropogenic factors and interannual variations of large-scale climate patterns (Drinkwater et al., 2010; Roessig et al., 2005). The latter shaping environmental variability and nutrient dynamics, thereby playing a prominent influence on the structure and functioning of plankton (Molinero et al., 2013). Understanding how climate interacts with these communities is therefore fundamental to develop adequate policies for a sustained use of marine ecosystems assets (Chust et al., 2013; Primo, 2012), as plankton constitute a major vector of energy transfer from primary productivity to fish.

Zooplankton communities have been useful to track climate-driven environmental changes (Hays et al., 2005). This is partly due to their pivotal role in marine food webs linking primary production with higher trophic levels, and to their non-linear responses face changing environmental conditions, which make them valuable sentinels of ecosystem changes (Hays et al., 2005; Taylor et al., 2002). In addition, these organisms are poikilothermic, and therefore not able to regulate internally environmental temperature. Hence, temperature changes in the marine environment directly affect their fundamental functional processes, such as ingestion, respiration and reproductive development (Mauchline, 1998).

Among zooplankton taxa, gelatinous carnivore's species (e.g., medusa, ctenophores and siphonophores) have an important role in food web dynamics by shaping top-down and bottom-up controls (Hosia et al., 2014; West et al., 2009), through predation pressure and modifying nutrient cycles via excretion and organic matter decomposition (Condon et al., 2011; Pitt et al., 2007; Ruzicka et al., 2012). Gelatinous zooplankton organisms are provided with a suite of attributes that enable them surviving in disturbed marine ecosystems and recovering rapidly as conditions improve (Richardson, 2008). In recent years, extensive outbreaks of gelatinous plankton have been recorded in several estuarine and coastal waters raising concerns about potential changes in the entire pelagic ecosystem dynamics (Lucas et al., 2014). However, despite the increasing global interest on these events, knowledge gaps remain in regards to the underlying factors driving the abundance changes of these organisms (Condon et al., 2014). In this study, we assess interannual and seasonal changes of gelatinous carnivore zooplankton in the Mondego estuary over the period 2003 to 2013. The western coast of the Iberian Peninsula, where the study area is located, is one of the four major upwelling regions in the world. This seasonal phenomenon mainly occurs during the spring−summer season and is shaped by the anticyclonic activity (Alvarez et al., 2008; Pérez et al., 2010; Santos et al., 2011), promoted by the positive phase of the North Atlantic Oscillation (NAO) (Lynam et al., 2004; Santos et al., 2011).

Here we examine the potential cascading effects of large-scale atmospheric phenomena to local hydrography and to changes in abundance and structure of the gelatinous carnivore zooplankton in the Mondego estuary. We hypothesized that climate signals shape inter-annual abundance changes of these organisms through their influence on regional weather patterns and the upwelling activity, which in turn shape local hydrographic conditions, eventually promoting favorable conditions for gelatinous carnivore zooplankton. We quantified these relationships by using a structural modelling approach, and further assessed seasonal changes in gelatinous carnivores at the community and species levels.

2.4 Materials and methods

2.4.1 Study area

The Mondego Estuary (40°08’N, 8°50’W) is situated in a warm temperate region on the west cost of Portugal (Fig. 2.1), where the atmospheric variability is strongly influenced by NAO (Trigo et al., 2004). It is an intertidal and shallow system composed by two arms (north and south) divided by Murraceira Island and characterized by different hydrological characteristics. The northern arm is deeper (4-8 m deep at high tide), represents the principal navigation channel and directly connects with Mondego River. The southern arm is shallower (2-4 m deep at high tide), is more silted up than the northern arm, the navigation mainly depends on the tides and on the fresh flow inputs from a small tributary, the Pranto River, whose discharges are controlled by a sluice (Cardoso et al., 2004; Grilo et al., 2012). A full and complete description of the system can be found in Marques et al., (2005).

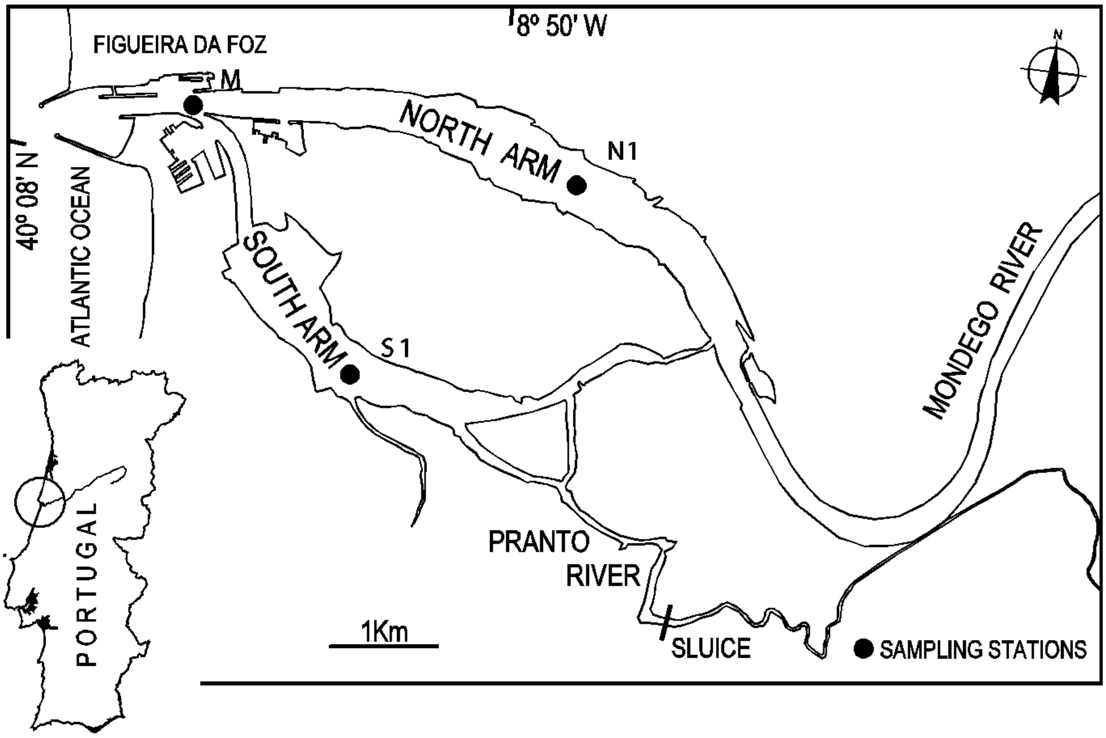

Figure 2.1: Location of sampling stations (M: Mouth; S1: Southern arm; N1: Northern arm) in the Mondego estuary.

2.4.2 Biological data

The gelatinous carnivores' organisms were collected monthly from January 2003 to December 2013 at three sampling stations in the Mondego Estuary (M – mouth; N1 – Northern Arm; S1 – Southern arm) (Fig. 2.1). Samples were taken by subsurface horizontal tows, using a plankton net (open diameter 0.5 m, mesh size 335 µm, tow speed: 2 knots, tow length: 3 minutes, 1 replicate plankton tow per station). The volume of water filtered was estimated by a Hydro-Bios flow meter fixed in the opening of the net (the volume filtered averaged 39±22 m^3). Then, organisms were immediately fixed with 4 % buffered formalin, transferred to 70 % ethanol in the lab and separated under dissecting microscope. Gelatinous organisms were identified, whenever possible, to the lowest taxonomic level and abundance was expressed as number of organisms per cubic meter (ind.m^3). Regarding siphonophores, only nectophores of *Muggiaea atlantica* and *Muggiaea kochii* were counted and used in data analysis.

2.4.3 Physical data

We used the North Atlantic Oscillation and regional atmospheric variability to assess the climate influence experienced by the Mondego estuary. The NAO is a pattern of atmospheric circulation characterized by cyclical oscillations of the difference in sea level pressure between Iceland and Azores, that influences the weather system over the North Atlantic, North Sea, and Europe (Hurrell, 1995). The NAO mixes up atmospheric mass between Artic and subtropical Atlantic and affects the ocean through modifications in salinity, gyre circulation, and surface air temperature (Hurrell, 1995; Hurrell et al., 2003). In addition, we used Upwelling Index data (UI) provided by the Instituto Español de Oceanografía. Upwelling is an oceanographic phenomenon that involves wind-driven motion of cooler, nutrient-rich water toward the ocean surface, promoting higher primary production. Positive (negative) UI values mean upwelling (downwelling) conditions (Santos et al., 2011). This index corresponds to the upwelling activity in the offshore area relative to the Mondego Estuary (Figueira da Foz region) and was computed by the Meteogalicia WRF atmospheric model (http://www.wrf-model.org). At each sampling event the water temperature, salinity, pH and dissolved oxygen were measured with appropriate sensors (WTW). Data belong to the Mondego Estuary survey headed by the Centre of Functional Ecology, University of Coimbra.

2.4.4 Statistical analysis

To explore the potential effect of hydroclimate influence on the interannual variations of gelatinous organisms, a five-step procedure was used.

First, time series were standardized to zero mean and unit variance. Then, linear regressions were performed to remove temporal trends and residual values were retained for analysis.

Second, to describe dominant patterns of hydrographic variability in the Mondego estuary, it was applied a Principal Component Analysis (PCA) on a matrix, Z, composed by the hydrological records. This allows integrating in few variables the climate variability. The first principal component (PC1) accounted for 61% of the hydrological variance and was used as proxy of local environmental conditions.

Third, the relationship between the NAO and the abundance of gelatinous carnivores on a monthly scale was quantified. As climate signals are non-stationary wavelet analysis (Continuous Wavelet Transform, CWT) was used to assess the time-varying signal of each time series and then was also used the wavelet coherence method to quantify their correlation in the time frequency space (Grinsted et al., 2004). Continuous Wavelet Transform performs a local time-scale decomposition of time series quantifying its spectral characteristics as a function of time (Cazelles et al., 2008; Hidalgo et al., 2011). The Morlet wavelet function was used as it better describes time series with unknown frequencies and allows a better separation of the phase and the amplitude of the studied signal (Cazelles et al., 2008; Percival and Walden, 2000). The 5% statistical significance level was determined by using bootstrap simulations (1000 times) considering a first order autoregressive process with lag-1 autocorrelation. The statistical significance was assessed relative to the null hypotheses that the signal is generated by a stationary process, i.e. mean and variance of the time series do not vary with time (Percival and Walden, 2000). Then, from the CWT of each two climate phenomenon, the wavelet coherence method was performed, to identify areas with high common power and significant links in the time frequency space between the two phenomena. To graphically display the temporal relationship only data within the cone of influence was used.

Fourth, partitioning effects of external forces driving the overall variability of gelatinous carnivores were assessed and quantified using structural equation modelling (SEM), which allowed depicting direct and indirect effects, and their relative importance. The strength and sign of links and the quantification of the model structure were determined by simple and partial multivariate regression and Monte Carlo permutation tests (1000 replicates), while the Bayesian Information Criterion (BIC) and Chi-square values were used to assess robustness and fit of the overall path model (Alsterberg et al., 2013). The individual path coefficients (i.e. partial regression coefficients) indicate the strength of the relationship between causal and response variables.

Fifth, heat diagrams were used to picture both seasonal and interannual abundance changes of gelatinous carnivore zooplankton. To do so, data were standardized to zero mean and unit variance, and the range of values was quantile divided. To each quantile a specific colour was assigned from light grey (quantile .10) to dark grey (quantile .90), representing low and high values, respectively. In addition, abundance changes were detected using the cumulative sum of standardized ordinary least square residuals (OLS-based CUSUM test). This technique allows for computing the probability of significant modifications in a time-series, and is useful in detecting shifts in chronological records (Fernández De Puelles and Molinero, 2013). After identifying the main periods characterizing the interannual variability of the gelatinous carnivore community, we examined the seasonal changes by computing the mean annual cycle prior and after 2007. Statistical differences between mean annual cycles during each period were tested by using a Kruskal-Wallis test. Furthermore, we assessed the interannual changes of the timing of seasonal peak; we centred Julian days to the median of the month in which the first annual peak was observed, i.e. abundance >30 ind.m^3. Thus, Julian days are centred to day 15 of each month (i.e. if the peak occurred in March the corresponding Julian day is March 15 = Julian day 74). The interannual variations of the timing of seasonal peak were regressed linearly against temperature to quantify their relationship.

Lastly, species-specific changes were computed. To do so, only species showing abundance larger than 0.1% in the whole community were used. A list of 28 species was selected and heat maps were built to show species year-to-year changes throughout the period examined.

2.5 Results

2.5.1 Gelatinous carnivore zooplankton abundance and composition

Predominant species of gelatinous carnivores in the Mondego estuary were mainly represented by hydromedusae and siphonophores, in particular by 20 taxa of hydromedusae and 2 taxa of siphonophores (Fig. 2.2 and Table 2.1).

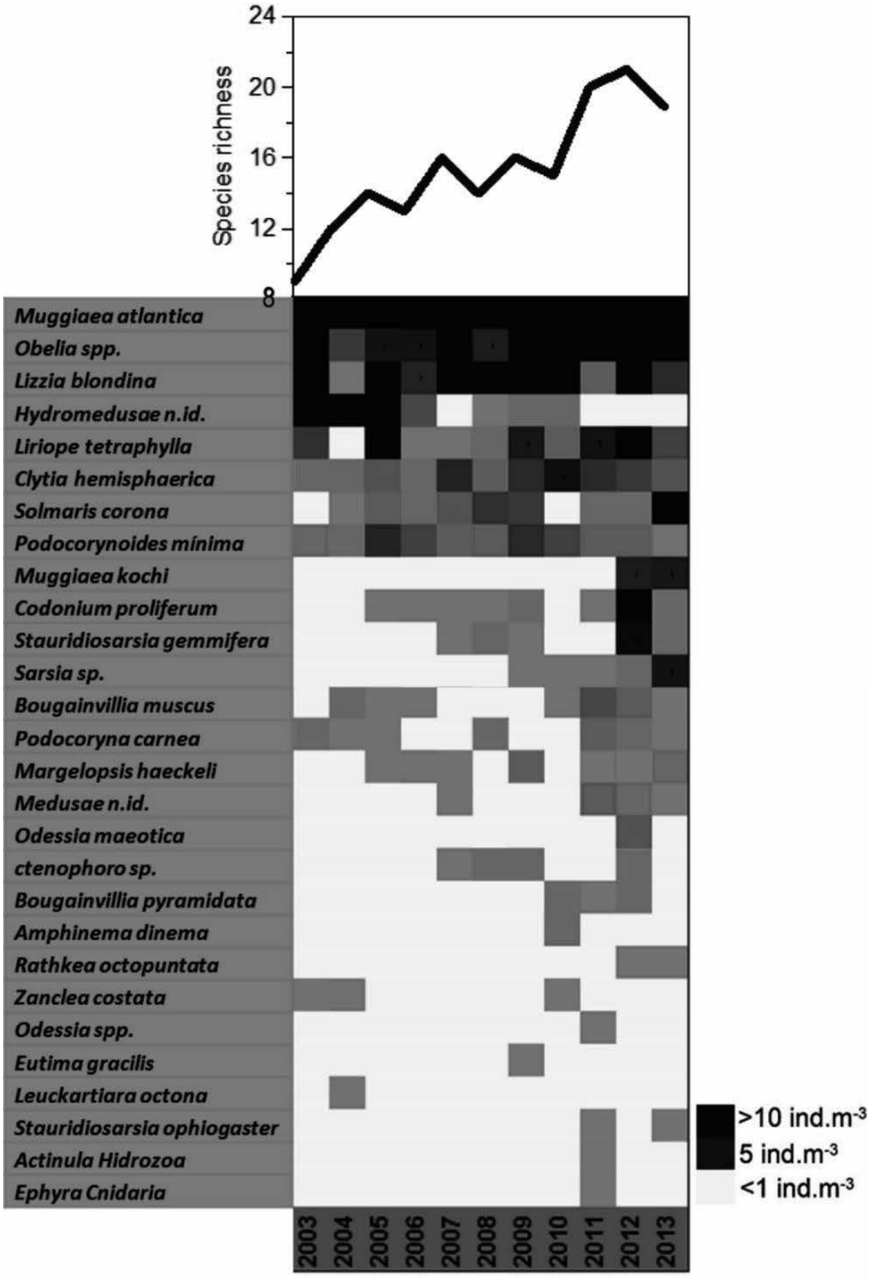

Figure 2.2: Temporal variations and species richness of gelatinous carnivores' species throughout the period investigated. Notice that the years 2007-2008 rare species (< 1 Ind.m^{-3}) increased in abundance.

Table 2.1: Species list and mean abundance (± standard deviation) throughout the period investigated.

Taxa	**(2003-2007)**		**(2008-2013)**	
	Mean	**St. Dev.**	**Mean**	**St. Dev.**
Muggiaea atlantica	9,0877	46,5256	21,2620	53,8679
Lizzia blondina	2,1398	9,5545	1,0166	2,9987
Obelia spp.	1,7595	7,4947	1,8448	5,3315
Hydromedusae n.id.	1,0224	3,7522	0,0159	0,1021
Liriope tetraphylla	0,4138	2,6960	0,4562	1,8436
Podocorynoides minima	0,1928	1,1374	0,1892	0,6317
Clytia hemisphaerica	0,1608	0,7514	0,3536	1,2538
Solmaris corona	0,0727	0,2737	0,2866	1,5116
Codonium proliferum	0,0449	0,2664	0,0676	0,3657
Bougainvillia muscus	0,0116	0,0592	0,0610	0,4084
Podocoryna carnea	0,0099	0,0968	0,0296	0,1683
Margelopsis haeckeli	0,0039	0,0379	0,0340	0,1927
Zanclea costata	0,0023	0,0187	0,0004	0,0059
Medusae n.id.	0,0010	0,0132	0,0355	0,1465
Stauridiosarsia gemmifera	0,0010	0,0102	0,1429	1,5503
ctenophoro sp.	0,0008	0,0107	0,0175	0,1174
Leuckartiara octona	0,0007	0,0091	0,0000	0,0000
Odessia spp.	0,0000	0,0000	0,0011	0,0161
Rathkea octopuntata	0,0000	0,0000	0,0045	0,0307
Odessia maeotica	0,0000	0,0000	0,0318	0,3547
Stauridiosarsia ophiogaster	0,0000	0,0000	0,0005	0,0046
Sarsia sp.	0,0000	0,0000	0,1255	1,1239
Muggiaea kochi	0,0000	0,0000	0,2027	1,0027
Eutima gracilis	0,0000	0,0000	0,0008	0,0119
Ephyra Cnidaria	0,0000	0,0000	0,0002	0,0022
Bougainvillia pyramidata	0,0000	0,0000	0,0125	0,0941
Amphinema dinema	0,0000	0,0000	0,0097	0,1316
Actinula Hidrozoa	0,0000	0,0000	0,0002	0,0029

During the 11-year time series, *Obelia* spp., *Lizzia blondina, Clythia hemispaerica, Liriope tetraphylla and Solmaris corona* were the five dominant hydromedusae species, whereas *Muggiaea atlantica* was the dominant siphonophore species (Fig. 2.2 and Fig 2.3). *M. atlantica* showed the higher abundances in July 2009, whereas lower abundances were found in December 2008 (Fig. 2.3). *Obelia* spp. and *Lizzia blondina* showed higher abundance values in 2003. In subsequent years, their presence in the Mondego estuary has been constant, with seasonal high abundance peaks (Fig. 2.2 and 2.3). *Liriope tetraphylla* presented maximum abundance in 2005, followed by a decrease until 2009, when its abundance increased again (Fig. 2.2 and

2.3). During the period investigated, *Clythia hemisphaerica* occurred every year, showing an increase of the seasonal abundance after 2008, reaching the maximum value in 2012 (Fig. 2.2 and 2.3). Lastly, *Solmaris corona* was observed every year although in very low abundances and it was completely absent during 2003 and 2010; the major peak of this species was observed in 2013 (Fig. 2.2 and 2.3). Beyond *M. atlantica*, which showed every year abundance > 10 ind.m^{-3}, notice that from years 2007-2008 most of species increased in abundance (Fig. 2.2).

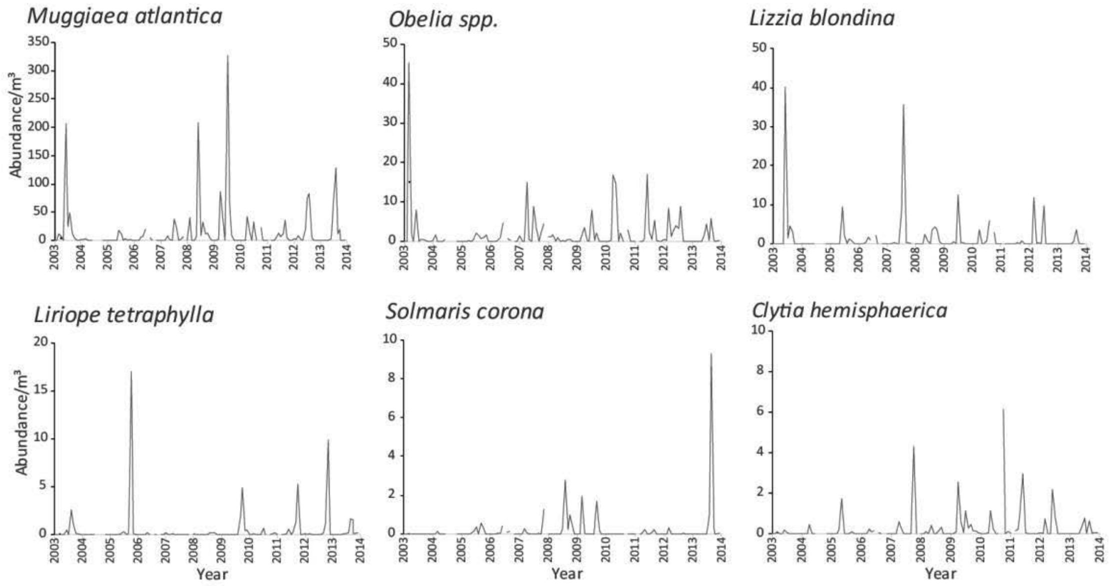

Figure 2.3: The mean monthly abundance species of gelatinous carnivores' organisms in the Mondego estuary during the period investigated.

2.5.2 Environmental variability

The NAO showed a marked variability at monthly and interannual scales (Fig. 2.4a). It was positive after 2008, although a dramatic drop occurred during the winters of 2010 and 2011, followed by a short, negative phase in 2013. Along with these changes, marked interannual variations were observed in the upwelling activity offshore the Mondego estuary, as indexed by the UI. The seasonal variability of the upwelling signal displayed a mean peak in summer, while interannual changes rose after 2007 and showed an absolute maximum late 2007 (Fig. 2.4b).

In turn, the aquatic environment in the Mondego Estuary displayed prominent monthly variations of hydrological conditions (Fig. 2.4c), that further exhibited larger variance around 2007-2010 (CUSUM test p<0.005) followed by a slight decrease. Likewise, the magnitude in their variance change was 2.5-fold higher after 2007. The governing temporal pattern of climatic and hydrological signals displayed concomitant changes after 2007. The NAO and regional climate exhibited prominent changes after 2007-2009 and 2010-2012, respectively (CUSUM test p<0.05).

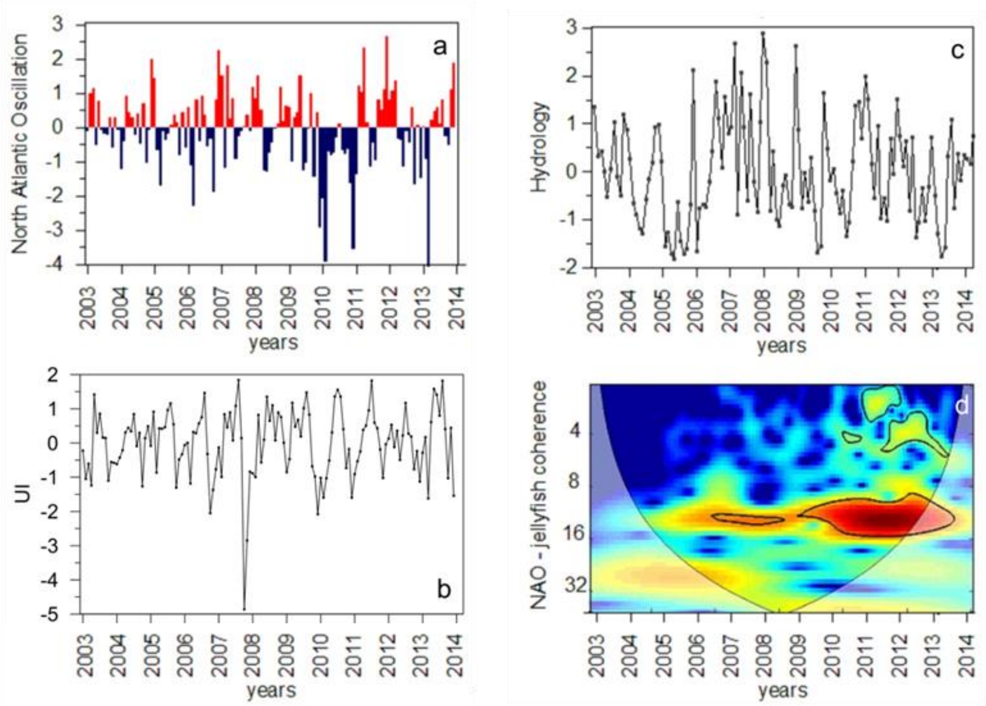

Figure 2.4: a: North Atlantic Oscillation. b: Upwelling Index in Figueira da Foz; c: Hydrological variability in the Mondego Estuary; d: Wavelet coherence between the North Atlantic Oscillation and the gelatinous carnivore's monthly changes.

2.5.3 Couplings between NAO, regional climate and gelatinous carnivore organisms' variability

The potential connection between the above climate changes, as indexed by the NAO and the variability of gelatinous carnivore zooplankton was assessed by means of wavelet analysis (Fig. 2.4d). Results showed a discontinuous interaction between climate and gelatinous carnivores that is, prior 2007 the relationship was elusive, while a significant increase arose afterwards, as pointed out by their wavelet coherence. It is worth noticing that the maximum coherence came along with the largest hydroclimate forcing, i.e. larger variance of climate and hydrology, which translated into a larger marine influence into the Mondego estuary system. The partitioning effects through the SEM unveiled cascading influences from NAO and

UI on local hydrology, ultimately influencing the overall temporal pattern of gelatinous carnivore zooplankton in the Mondego estuary (Fig. 2.5). The atmospheric forcing promoted by the NAO was closely linked with regional climate variability (path coefficient= 0.68), as well as with interannual changes in UI (path coefficient=0.33). These changes permeated local hydrological conditions affecting salinity (path coefficients= 0.25 and 0.28, UI and regional climate respectively), and temperature (path coefficients= -0.18 and 0.22, UI and regional climate respectively). In turn, temperature showed closely linked with changes in gelatinous carnivore zooplankton (path coefficient= 0.54) acting as a mediator factor between large, i.e. NAO, and regional, i.e. UI, scale forcing processes with estuarine ecological changes.

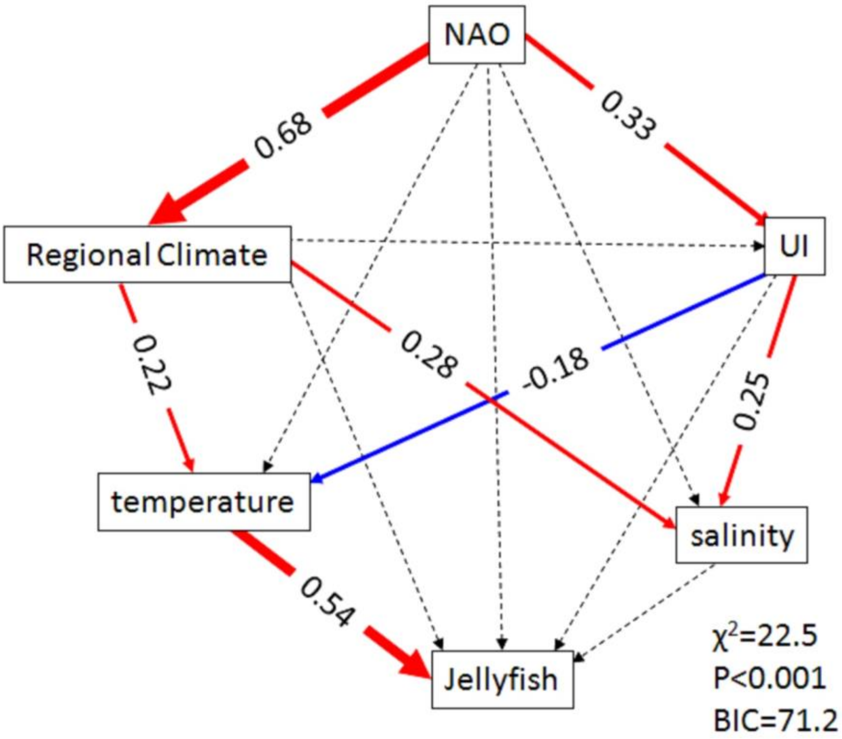

Figure 2.5: Path diagram showing direct and indirect effects of climate-related forcing on the temporal variations of gelatinous carnivores' organisms. Red paths are statistically significant ($p < 0.01$), whereas dashed lines are not.

2.5.4 Interannual and seasonal changes of gelatinous carnivore zooplankton in the Mondego estuary

In the light of the above climate-related environmental changes, we portrayed the interannual and seasonal patterns of gelatinous carnivore variability (Fig. 2.6a, 2.6b). Superposed to the marked year-to-year changes, we found a major change late 2007 (CUSUM test $p<0.05$) (Fig. 2.6a), which corresponded to a conspicuous abundance increase of mean annual values. At the shorter time scale, noticeable changes occurred in the phenology of the group. For instance, we found an earlier timing of the seasonal peak after 2007, as well as a conspicuous increase in the species richness (Fig. 2.6b). These changes depicted modifications in the annual pattern prior and after 2007. During the period 2003-2007, the annual pattern showed a unimodal peak, in summer, as the main feature in the seasonal abundance changes. Afterwards, during the period 2008-2013, the mean seasonal abundance changes displayed a bimodal pattern, with major abundance in spring and summer. The former peak corresponding to the earlier phenology likely driven by enhanced spring temperatures (Fig. 2.6c). Indeed, the timing of the seasonal peak of gelatinous carnivores shifted earlier during high winter spring temperatures, while lower temperatures during the same period delayed the seasonal peak. The shift in the seasonal timing was ca. 122 days; that is, under high temperatures gelatinous carnivores peaked as earlier as March, while under low temperatures the peak occurred in July (Fig. 2.6d). Furthermore, during the years 2008-2013, we assisted an increase in the abundance of the most occurring species and further observed the insurgence of new species (Table 2.1).

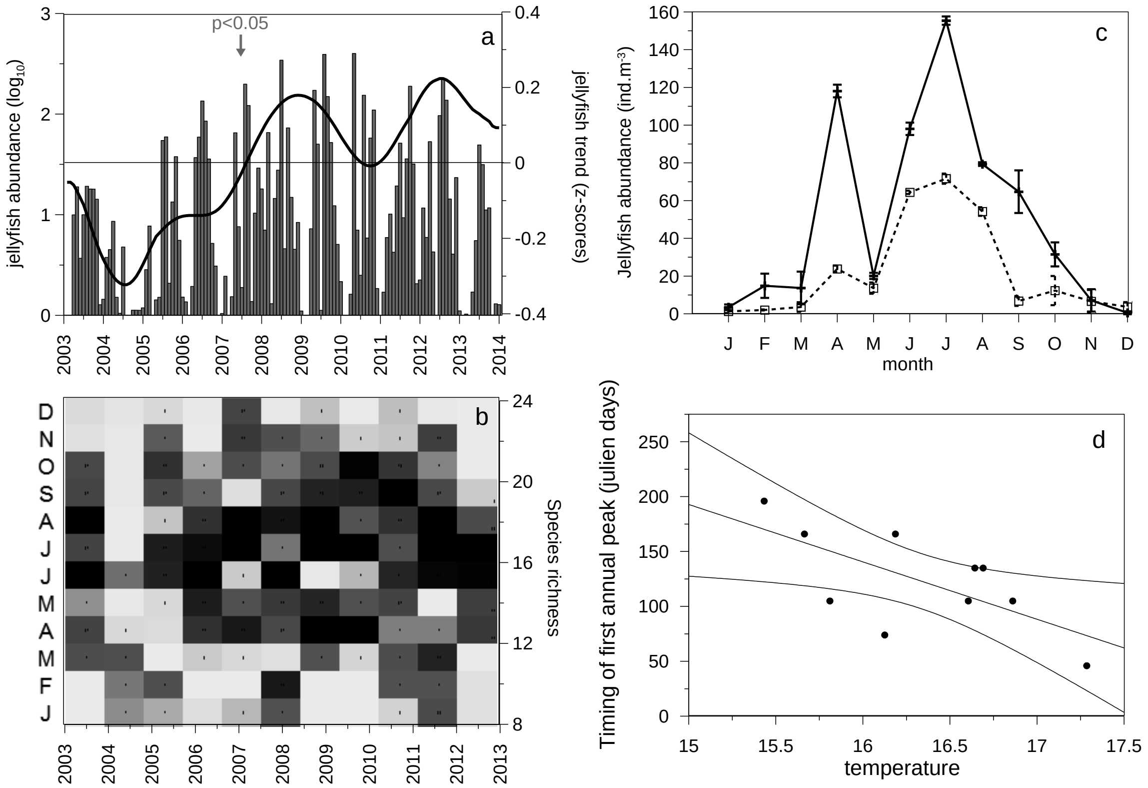

Figure 2.6: a: Interannual and b: seasonal changes of gelatinous carnivores abundance in the Mondego estuary; c: changes noticed in the mean annual cycle during the years 2003-2007 (dashed line) and 2008-2013 (continuous line); d: relation between the timing of seasonal peak of gelatinous carnivores expresses in julien days versus spring temperature.

2.6. Discussion

2.6.1 Climate variability and gelatinous carnivore zooplankton

Presently, evidence has been shown on the relationship between the abundance and distribution of gelatinous carnivore zooplankton and climate variability (Lynam et al., 2005; Molinero et al., 2008). The results presented here, however, pointed out that such a relationship displays a discontinuous interaction due to the non-stationary character of climate forcing (Fernández De Puelles and Molinero, 2013; Hidalgo et al., 2011; Molinero et al., 2013). The relation increases after 2007, along with higher variation of the upwelling activity and local hydrological conditions, which promoted the increasing trend in the abundance of gelatinous carnivores and species richness found over the period investigated. The observed changes of the NAO enhanced the variance in regional wind and precipitation, and the increase of the upwelling activity. A number of recent studies have pointed out the influence of upwelling events in the distribution pattern of gelatinous zooplankton (Miglietta et al., 2008; Pavez et al., 2010; Sanvicente-Anõrve et al., 2009). In addition, temperature has been also identified as one of the most important factors influencing temporal and spatial distribution patterns of gelatinous carnivore zooplankton (Blackett et al., 2014; Lucas et al., 2014; Pavez et al., 2010; Sanvicente-Añorve et al., 2009). Indeed, warm temperatures promote an increase of asexual reproduction in hydrozoans and, consecutively, an upsurge in the abundance of Hydromedusae species (Purcell, 2005), which may explain the earlier appearance of hydromedusae in the Mondego estuary during the second period, 2008-2013.

An additional explanation as possible driver of the observed changes in these organisms is the variation in freshwater flow. Recent studies pointed out the close connection between river discharge (river plume advection) and the increased presence of marine species in the Mondego estuary (e. g. Baptista et al., 2010; Marques et al., 2014; Primo et al., 2015, 2011). In concurrence with the observed changes, the same authors pointed out marked drought events in the Mondego estuary over the period 2003-2013. The increased prevalence of marine conditions in the estuary promote the entrance of marine species, including gelatinous carnivore zooplankton, which can alter the structure and dynamics of the estuarine community.

Current understanding of upwelling events influence on biological communities' structure points out the enhancement of primary production, as nutrients inputs from deep waters increase. Therefore, we also hypothesized that the mechanism linking NAO and gelatinous carnivore plankton in the Mondego estuary, includes changes in the primary production and reproduction of prey ultimately leading to local gelatinous zooplankton blooms (Drinkwater et al., 2010; Lynam et al., 2005).

2.6.2 Gelatinous carnivore zooplankton community in Mondego estuary

The siphonophore calycophorans *Muggiaea atlantica*, a typical boreal Atlantic species, constitute the bulk of the gelatinous carnivore community in the Mondego estuary, accounting for the annual maxima of the population. This species is widespread in inshore temperate waters, while a spring-summer peak appears as a common pattern in several inshore temperate waters (Blackett et al., 2015).

Siphonophores, particularly *M. atlantica,* are known as voracious planktivorous predators, and their growth rate tend to increase with increasing prey density, e.g. copepods (Purcell, 1982), the major constituent of zooplankton community. In this study, the temporal pattern of gelatinous carnivore zooplankton was consistent with the copepods and marine cladocera abundance as described by Marques et al. (2014). This jellyfish-copepod link was previously reported by Primo et al. (2012), which revealed significant relationship between summer gelatinous carnivore zooplankton community in Mondego estuary and the copepod *Acartia clausi*, the major constituent of the mesozooplankton community in Mondego ecosystem.

It is worth noting that the structure led by the species contribution to the total abundance is similar in the two identified periods, with *M. atlantica* as the dominant species throughout the study. The progressive expansion of the distribution of this species into some regions (Batistić et al., 2013; Blackett et al., 2014) suggests that this species is able to exploit favorable environmental conditions more efficiently than other siphonophores (Licandro et al., 2012).

As observed here, the relationship of gelatinous carnivore abundance with temperature evolves according to the strength of the hydroclimate forcing, with higher abundances concurrent with higher temperatures. It is worth noticing that the gelatinous carnivore zooplankton community changed, not only in terms of abundance or structure (i.e. diversity), but they also showed conspicuous phenological changes with the main seasonal peak appearing earlier in high winter-spring temperatures and shifting the annual pattern from unimodal (summer peak) to bimodal (spring and summer peaks). These phenological changes are in agreement with former studies showing that at small spatial scales, such as small estuarine systems, spring temperature is a main driver of gelatinous carnivore zooplankton blooms (Mills, 2001; Purcell et al., 2012; Robinson and Graham, 2014). The results obtained in this study showed a close connection between the atmospheric field playing out in the North Atlantic region and the hydrographic patterns around the Mondego estuary. This is the first study in the Iberian Peninsula, Portugal, identifying the effects of mounting winter-spring temperatures on the structure (species richness) and dynamics (phenology) of gelatinous carnivore zooplankton. There results provide baseline information for comparison with other temperate estuarine systems while elucidates major factors shaping plankton communities in the Iberian Peninsula.

2.7 Acknowledgements

The authors are indebted to all the colleagues that assisted in the field and lab work. We wish to thank anonymous reviewers and the Editor for their most helpful and constructive comments on the manuscript. The present work was supported by FCT (Portuguese Foundation for Science and Technology) through a PhD grant awarded to Mariaelena D'Ambrosio (SFRH/BD/91541/2012) and through COMPARE Project (PTDC/MAR/121788/2010) financed by POPH (Portuguese Operational Human Potential Program), QREN Portugal (Portuguese National Strategic Reference Framework), and MCTES (Portuguese Ministry of Science, Technology, and Higher Education). JCM gratefully acknowledge the European Commission (OCEAN-CERTAIN, FP7-ENV-2013-6.1-1; no: 603773).

CHAPTER 3

SPATIAL DISTRIBUTION AND SEASONAL PATTERNS OF THE SIPHONOPHORES *MUGGIAEA ATLANTICA* AND *MUGGIAEA KOCHII* IN A TEMPERATE ESTUARINE ECOSYSTEM

3.1 Abstract

In the last decades, the massive proliferations of gelatinous zooplankton, as well as its higher prevalence, reported in some coastal areas and temperate seas are indicative of significant changes in the functioning of marine and estuarine ecosystems. In this study, the distribution and abundance of calycophoran siphonophores *Muggiaea atlantica* and its congeneric *Muggiaea kochii* were analysed during two years (2014 and 2015) in Mondego estuary (Portugal), with the aim to explain how their distribution was influenced by different environmental factors, such temperature, salinity and prey availability. Samples were collected in three different zones of the estuary, from November 2013 to November 2015. Generally, *M. atlantica* was the most abundant, and both species nectophores and gonophores showed increased densities in the downstream areas. In 2015, an increase of *M. atlantica* nectophores was observed, and both species displayed a shift in the annual occurrence pattern (from unimodal to bimodal peak). Salinity and prey were the main drivers affecting the abundance and distribution of siphonophores in the estuary, with increased influence of marine prey during 2015. This study represents a step towards a better knowledge of the biology and ecology of siphonophores.

3.2 Keywords

Gelatinous zooplankton, Siphonophorae, Calycophorae, Northwestern Atlantic Ocean, Mondego estuary.

3.3 Introduction

The hydrozoan class of siphonophores is constituted mainly by pelagic but also benthonic and neritic free-swimming colonial species (Mapstone, 2014; Pugh, 1974), representing one of the most abundant zooplankton carnivorous species worldwide (Carré and Carré, 1991; Pugh, 1989). They have a relevant ecological role because, under appropriate environmental factors, such as temperature, salinity and food availability, these organisms can rapidly reproduce, leading to seasonal large blooms (Blackett et al., 2015; Lucas et al., 2014). Siphonophores are voracious planktivorous predators that can significantly affect prey populations, e.g. copepods (Purcell, 1982), and the structure of marine communities (Blackett et al., 2015; Mills, 1995; Pitt et al., 2008).

The calycophoran siphonophores *Muggiaea atlantica* (Cunningham, 1892) and *Muggiaea kochii* (Will, 1844) are very abundant components in neritic waters of temperate regions, mainly in spring-summer (Angeles, 1971; Bouillon et al., 2004; Gili et al., 1987). Their life cycle comprises an asexual polygastric stage and a sexual eudoxid stage (Kirkpatrick and Pugh, 1984; Mackie et al., 1987); the eudoxids are free-swimming and release the gonophores for several months that, via external fertilisation, generate a new polygastric colony (Carré and Carré, 1991; Mapstone, 2014). *Muggiaea kochii* is a very common Mediterranean species (Batistić et al., 2013), with a predilection for relative high-temperature waters (>13ºC) (Batistić et al., 2013). It is also present in several neritic Atlantic systems (Bouillon et al., 2004; Carré and Carré, 1991; Mapstone, 2014). In the Gulf of Mexico and in the Southern Brazilian Bight it is considered a dominant gelatinous species (Martell-Hernández et al., 2014; Nagata et al., 2014), whereas in the English Channel is described as a transient non-resident species (Blackett et al., 2015). *Muggiaea atlantica* is a typical Atlantic species (Bouillon et al., 2004 and references therein), considered as one of the major components of the neritic gelatinous zooplankton (Mapstone, 2014), and is considered a cool-temperate species, able to complete its life cycle at water temperatures below 13ºC (Batistić et al., 2013). In response to hydroclimatic changes since the 80's, *M. atlantica* has progressively colonized the Western Mediterranean, and more recently displaced the population of *M. kochii* in the

Adriatic Sea (Batistić et al., 2007). Besides, it has established new populations in the Western English Channel (Blackett et al., 2014) and in the German Bight, where it became the dominant carnivore species (Greve, 1994).

In the Mondego estuary, a temperate shallow system in the western Iberian coast, the gelatinous community is mainly composed by hydromedusae and siphonophores, being *M. atlantica* the bulk of the gelatinous carnivorous community (D'Ambrosio et al., 2016; Marques et al., 2006; Primo et al., 2012, 2009). The first record of *M. atlantica* in the Mondego estuary dates back to 1994 (Azeiteiro et al., 1999). Recently, D' Ambrosio et al. (2016) pointed out its decrease in the estuary from 2004 to 2009, when its abundance bloomed again. However, *M. kochii* has been considered a rare species in the estuary until 2012, after which its abundance also increased, becoming a common gelatinous species in the estuary (D'Ambrosio et al., 2016).

Several studies have already shown the importance of the relationship between the abundance and distribution of siphonophores and local oceanographic conditions in different areas (Lopez-Lopez et al., 2013; Palma and Silva, 2006; Pavez et al., 2010). It is worth noticing the influence of North Atlantic climate variability on the gelatinous carnivore zooplankton (Lynam et al., 2005). In the Mondego estuary, such control was more evident after 2007, when the North Atlantic Oscillation (NAO) and upwelling activity showed a larger variance, causing a shift in the abundance and the phenology of gelatinous zooplankton (D'Ambrosio et al., 2016). However, siphonophore annual patterns in temperate shallow systems, such as the Mondego estuary, where their increasing abundances can provoke important alterations in the ecosystem (Greve, 1994; Batistic et al, 2013), are still poorly known. In addition, there is a lack of studies about the basic ecological conditions (e.g.: temperature and salinity) ranges for their sexual reproduction in natural environments, which is an essential knowledge for forecasting possible future invasions and establishment in new areas, as well as for modelling their abundances in the current global climate change scenario. In the Mondego estuary, recent studies pointed out how the prevalence of marine conditions promoted the entrance of marine species, including gelatinous carnivore zooplankton (e. g. Baptista et al., 2010; Marques et al., 2014; Primo et al., 2015, 2011). This recent climatic scenario has turned the Mondego estuary in an exceptional natural laboratory to study the reproductive activity and abundance responses of these two-important carnivore gelatinous species.

Therefore, the present study aims to increase the knowledge of the annual abundance patterns of the congeneric species *M. atlantica* and *M. kochii* in the Mondego estuarine ecosystem and the influence of the environmental condition in their natural development. For that, changes in abundance and spatial distribution of polygastric and eudoxid stages of both species during two consecutive years were analyzed and the relationships with local environmental conditions were investigated.

3.4 Materials and methods

3.4.1 Study site

The Mondego estuary is a temperate shallow estuary located in the western Atlantic coast of Portugal (40°08’N, 8°50’W), which divides at the terminal part into north and south arms that join again near the mouth (Fig. 3.1). The two arms are separated by the Murraceira Island and are characterized by different hydrological characteristics. The northern arm is deeper (4-8 m deep at high tide; residence time <1 day), representing the principal navigation channel and directly connected with Mondego River. The southern arm is shallower (2-4 m deep at high tide; residence time < 3 days), more silted up in the upstream areas, and the navigation mainly depends on the tides and on the discharges of freshwater from a small tributary, the Pranto River, which discharges are controlled by a sluice (Cardoso et al., 2004; Marques et al., 2006). A full and complete description of the system can be found in Marques et al. (2005).

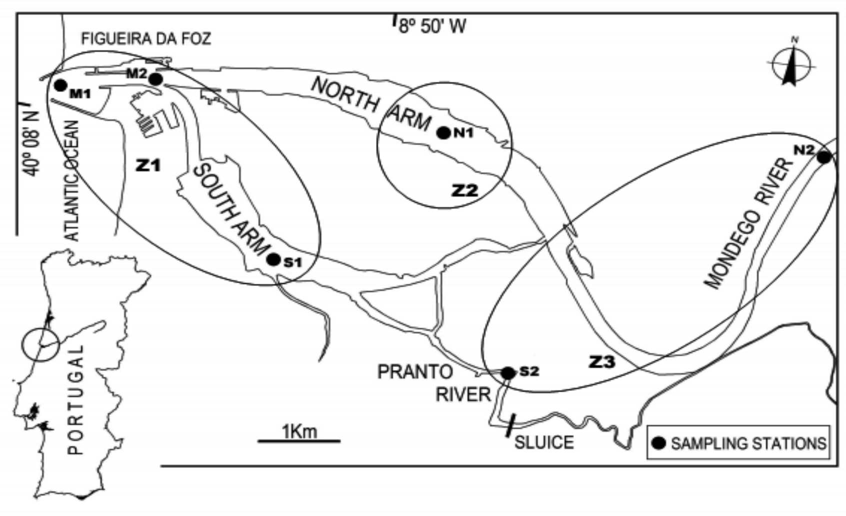

Figure 3.1: Geographical location of Mondego estuary, the six sampling stations (M1, M2, N1, N2, S1, S2) and their aggrupation in the three sampling zones (Z1, Z2 and Z3). More details about zonation are explained in the text.

3.4.2 Sample collection

Muggiaea species were collected once a month during the high tide from December 2013 until November 2015, at six sampling stations in the Mondego estuary (M1 e M2 – mouth; N1 e N2 – northern arm; S1 e S2 – southern arm) (Fig. 3.1), as a part of a long-term zooplankton research that began in 2003 (Marques et al., 2014). Samples were collected by subsurface horizontal tows horizontal (10 cm below surface), using a plankton net (open diameter 0.5 m, mesh size 335 µm,) and immediately fixed with 4 % buffered formalin. The volume of filtered water was estimated using a Hydro-Bios flow meter mounted in the opening of the net (the volume filtered per sample averaged 39±22 m^3). In the laboratory, siphonophores were separated under a dissecting microscope, identified and counted. Abundance was expressed as individuals per m^3.

Siphonophore colonies are very fragile and tend to break up into parts when captured by nets (Kirkpatrick and Pugh, 1984; Purcell, 1981). In the case of *Muggiaea* species, they only develop a single nectophore (Mackie et al., 1987), therefore *M. atlantica* and *M. kochii* anterior nectophores were counted to provide a direct estimation of their asexual polygastric stage abundance (Fig. 3.2a and 3.2b). The diphyids produce free-living eudoxids (sexual stage), which produce a succession of gonophores that ripen, liberate its gametes and detach one after the other for several months (Mackie et al., 1987; Mapstone, 2014). Each gonophore may be either male or female, and the same eudoxid may develop successively male or female gonophores, with an irregular alternation of the two (Carré and Carré, 1991). Since eudoxid stages of the genus *Muggiaea* are morphologically indistinguishable (Kirkpatrick and Pugh, 1984), we identified and counted female and male gonophores as a measure of the reproductive activity of both species of *Muggiaea* in the estuary (Fig. 3.2c and 3.2d).

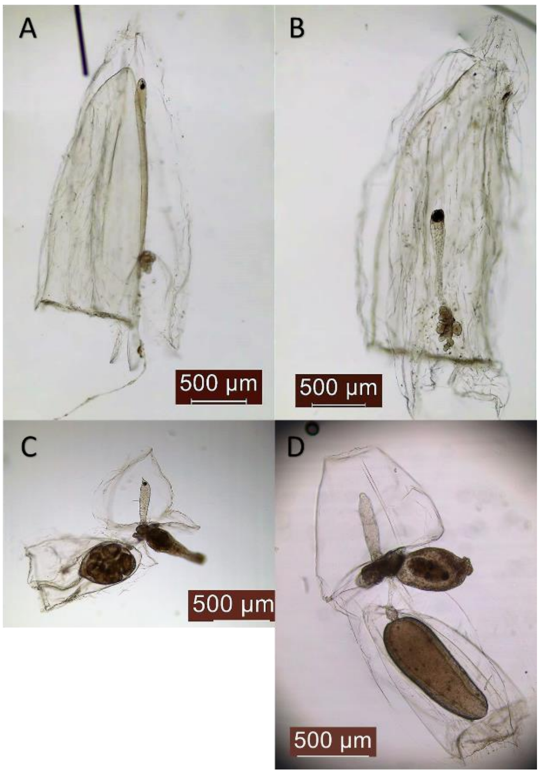

Figure 3.2: *Muggiaea* species pictures from the plankton samples. (A) *Muggiaea atlantica* anterior nectophore; (B) *Muggiaea kochii* anterior nectophore; (C) *Muggiaea* spp. eudoxid with a female gonophore; (D) *Muggiaea* spp. eudoxid with a male gonophore. (Photographs by João Neves).

Besides siphonophores, zooplankton samples were also collected by subsurface horizontal tows, using a plankton net (open diameter 0.5 m, mesh size 200 µm), following the same sampling procedure explained for siphonophores, in order to estimate potential prey abundances. Based on the literature (Blackett et al., 2015; Purcell, 1983, 1981), we selected different copepods groups and cirripedia (nauplius and cypris stages) as potential prey of *M. atlantica* and *M. kochii.*

The surface environmental variables temperature and salinity were recorded *in situ* simultaneously at each zooplankton sampling using a WTW Cond 330i. Precipitation data for the area were obtained from the weather station IFIGUEIR5 (www.wunderground.com) situated 4.5 km from the study area. The average precipitation data between 1981 and 2010 for central Portugal were acquired from the national Instituto Português do Mar e da Atmosfera (www.ipma.pt).

3.4.3 Statistical analysis

Based on the similarity of salinity (one-way ANOVA, $p < 0.001$), stations were grouped in three different zones: Z1, mouth and middle south arm (M1, M2, S1), characterized by salty waters; Z2, middle north arm (N1), the transition zone influenced by the seawater and the freshwater discharges from River Mondego; and Z3, the upper estuary (N2 and S2), characterized by the lowest salinity values (Fig. 1).

The relationships between environmental conditions and *Muggiaea* spp. abundances were tested with generalized linear models (GLMs). Models tested whether the independent variables of water temperature, salinity, precipitation and prey items concentrations were significant predictors for the log (x+1) transformed densities of *M. atlantica* and *M. kochii* nectophores and *Muggiaea* spp. female and male gonophores, separately. Prey items abundances were also log (x+1) transformed. Tests were performed for each year, individually, and the variable Zone was included in the models. The collinearity among predictor variables was evaluated by pair-wise (Pearson) correlation coefficients (r) and variables with r-values > 0.8 were removed. Model fit was assessed using Akaike's Information Criteria (AIC), and the model with lower number of significant predictor variables was considered the best fitted model. The statistical analyses were performed using R 2.10.0 (R Core Team, 2014).

3.5 Results

3.5.1 Environmental parameters

During the period investigated, water temperature followed a similar seasonal pattern in the three zones (Table 3.1; Fig. 3.3A), presenting the highest values during summer (Z3 on July 2015, 25.6 ºC) and the lowest in winter (Z2 on February 2014, 11.1 ºC) (Table 3.1; Fig. 3.3A). Z1 and Z2 had very similar values, whereas Z3 showed quite higher values during summer and slightly lower values in winter.

Table 3.1: Mean ± SD and minimum-maximum range values of temperature and salinity registered in Mondego estuary during the period investigated in the three different sampling zones.

	2014			2015		
	Z1	**Z2**	**Z3**	**Z1**	**Z2**	**Z3**
Temperature (ºC)						
Mean±SD	16±3	16±3	19±5	17±2	17±2	19±5
Min-Max	11.4-20.8	11.1-21	11.5-25	13.2-21	13-21	11.9-26
Salinity						
Mean±SD	23±11	19±13	10±8	31±3	30±4	17±6
Min-Max	0.7-39	0-38	0-21	25-34	22.7-34	7.4-28

Precipitation in Mondego area showed the typical seasonal variability, higher during autumn and winter months and lower during summer (Fig. 3.3B). Compared to the mean precipitation regime for central Portugal during 1981-2010, the year of 2014 corresponded to a normal precipitation year while 2015 was a dry year, especially during the winter. In 2014, the highest precipitation values occurred during winter (February, 123.7 mm) and autumn (November, 101.8 mm) and the lowest was recorded in summer (August, 2.2 mm), whereas in 2015, the maximum peaks were during spring (April, 54.3 mm; May, 51.9 mm) and autumn (October, 94 mm) and the lowest was recorded in summer (July, 0.5 mm).

The salinity annual trend in the estuary presented clear differences between the two studied years (Fig. 3.3C; Table 1). In 2014, two abrupt low salinity events, reaching values of zero in any of the three zones, occurred in winter (January-March) and autumn (November) while the maxima was during summer (August, 39.05). However, in 2015 zero values were never reached and a more stable salinity scenario was found in the estuary. The lowest values occurred during winter (February, 7.4) and the maxima in summer (May and July, 34.1) and autumn (September and November, 33.9) (Table 3.1).

The zones Z1 and Z2 showed similar values along the studied period, being Z2 more fluctuating than Z1. The zone Z3 always presented lower values than the other two zones (Fig. 3.3C; Table 3.1).

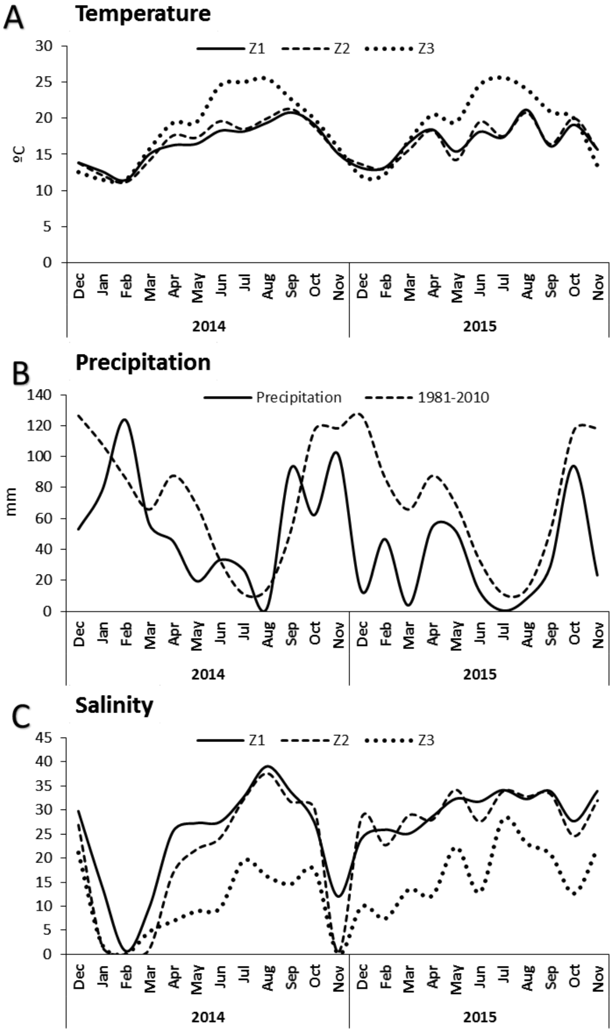

Figure 3.3: Temporal variation of the environmental parameters in Mondego estuary from December 2013 to November 2015. (A) Surface temperature in the three different sampling zones. (B) Precipitation values for the area (black line) and mean precipitation for the 1981-2010 period (dotted line). (C) Surface salinity values in the three different sampling zones.

3.5.2 Temporal and spatial variation of *M. atlantica* and *M. kochii* nectophores

Figure 3.4 and table 3.2 represent the spatial and temporal fluctuations in abundance of *M. atlantica* and *M. kochii* nectophores and *Muggiaea* spp. female and male gonophores. Only for graphical purposes, abundance data were log-transformed and standardized to zero mean and unit variance to reduce data variance.

Nectophores of both *Muggiaea* species followed similar seasonal trends, with the highest abundance values during the summer period and the lowest during the winter. Differences between both years were observed: in 2014 the abundance followed a bimodal trend with maximum peaks in early summer (June) and, secondly, in autumn (October), while in 2015 the general trend was unimodal for both species with the highest peak in summer (July) (Fig. 3.4).

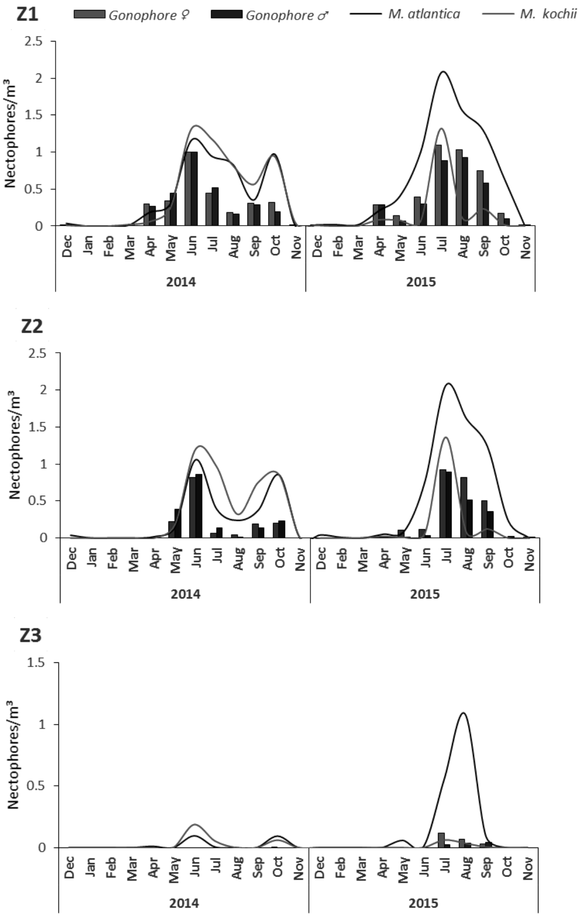

Figure 3.4: Temporal fluctuations in the abundances of *M. atlantica* and *M. kochii* nectophores (lines) and *Muggiaea* spp. female and male gonophores (columns) in the three different sampling zones from December 2013 to November 2015. To better appreciate the monthly distribution, data were log-transformed.

In 2014, both species presented similar monthly abundances except for summer and late autumn months when *M. kochii* was more abundant, resulting in a higher annual mean abundance for that species (Fig. 3.4, Table 3.2). However, in 2015, *M. atlantica* clearly dominated over its congeneric species all along the year, showing very high annual mean densities when compared to 2014 (Fig. 3.4, Table 3.2).

Spatially, Z1 and Z2 presented similar *Muggiaea* nectophores abundance, with Z1 usually displaying the maximum abundance values. In contrast, Z3 always showed the lowest abundances. The three zones showed the same annual trends, with the maximal abundance peaks in the same months, although with longer absence periods towards the upper estuary area (Z3) (Fig. 3.4).

Table 3.2: Mean ± SD and Maximum abundance values of *M. atlantica* and *M. kochii* nectophores and *Muggiaea* spp. male and female gonophores (Ind. m^{-3}).

	2014			2015		
	Z1	**Z2**	**Z3**	**Z1**	**Z2**	**Z3**
Nectophore *M. atlantica*						
Mean±SD	2.95±4.25	1.60±3.02	0.04±0.09	18.78±35.06	17.68±34.59	1.28±3.2
Max	13	10	0	119	115	11
Nectophore *M. kochii*						
Mean±SD	3.89±6.06	2.74±4.44	0.07±0.15	2.15±5.91	2.28±6.60	0.02±0.04
Max	20	15	1	20	22	0
***Muggiaea* spp. gonophore** ♀						
Mean±SD	1.21±2.31	0.58±1.44	0	2.91±4.08	1.59±2.59	0.04±0.09
Max	9	6	0	12	7	0
***Muggiaea* spp. gonophore** ♂						
Mean±SD	1.24±2.38	0.7±1.64	0	1.94±2.71	1.06±2.05	0.02±0.04
Max	9	6	0	7	7	0

3.5.3 Temporal and spatial variation of *Muggiaea* spp. gonophores

Both male and female gonophores of *Muggiaea* spp. presented similar seasonal trends and they basically followed the nectophores trend, with maximum peaks in summer and minimum or absent during winter (Fig. 3.4). They also presented a bimodal trend in 2014 and unimodal in 2015, with the same main maximum peaks as nectophores. Both male and female gonophores presented similar abundances in 2014, however female gonophores were more abundant in 2015.

Spatially, Z1 always presented the highest *Muggiaea* spp. gonophores abundance, Z2 the intermediate abundance and Z3 the lowest abundance, where they were virtually absent in 2014. Zones Z1 and Z2 showed the same annual trends but with longer absence periods in Z2. In the upper estuary area, Z3, only a few female gonophores were found in 2014 while both female and male gonophores, but more abundantly females, were present in summer and early-autumn months of 2015 (Fig. 3.4, Table 3.2).

3.5.4 Temporal and spatial availability of the potential prey

Figure 5 shows the seasonal variability of the density (percentage and Ind. m^{-3}) of the different *Muggiaea* spp. potential prey, during 2014 and 2015, in the three zones. During the period investigated, calanoids and cyclopoids were the most abundant groups (Fig. 3.5). Summer was characterized by higher densities of prey, mainly calanoids and cyclopoids, while lower abundances and a higher predominance of cyclopoids were observed in the winter.

Z3 zone displayed the highest prey abundance, largely constituted by calanoids, while zones Z1 and Z2 had lower abundances and more variability of groups, such as cyclopoids, harpacticoids and poecilostomatoids (Fig. 3.5).

Comparing both years, 2015 was characterized by an increase in abundance of the marine groups, e.g. harpacticoids, poecilostomatoids and cirripedia, and this was observed mostly during winter months (Fig. 3.5). Moreover, in Z1 the total abundance of copepods was higher in 2014, whereas in Z2 and Z3 increased in 2015.

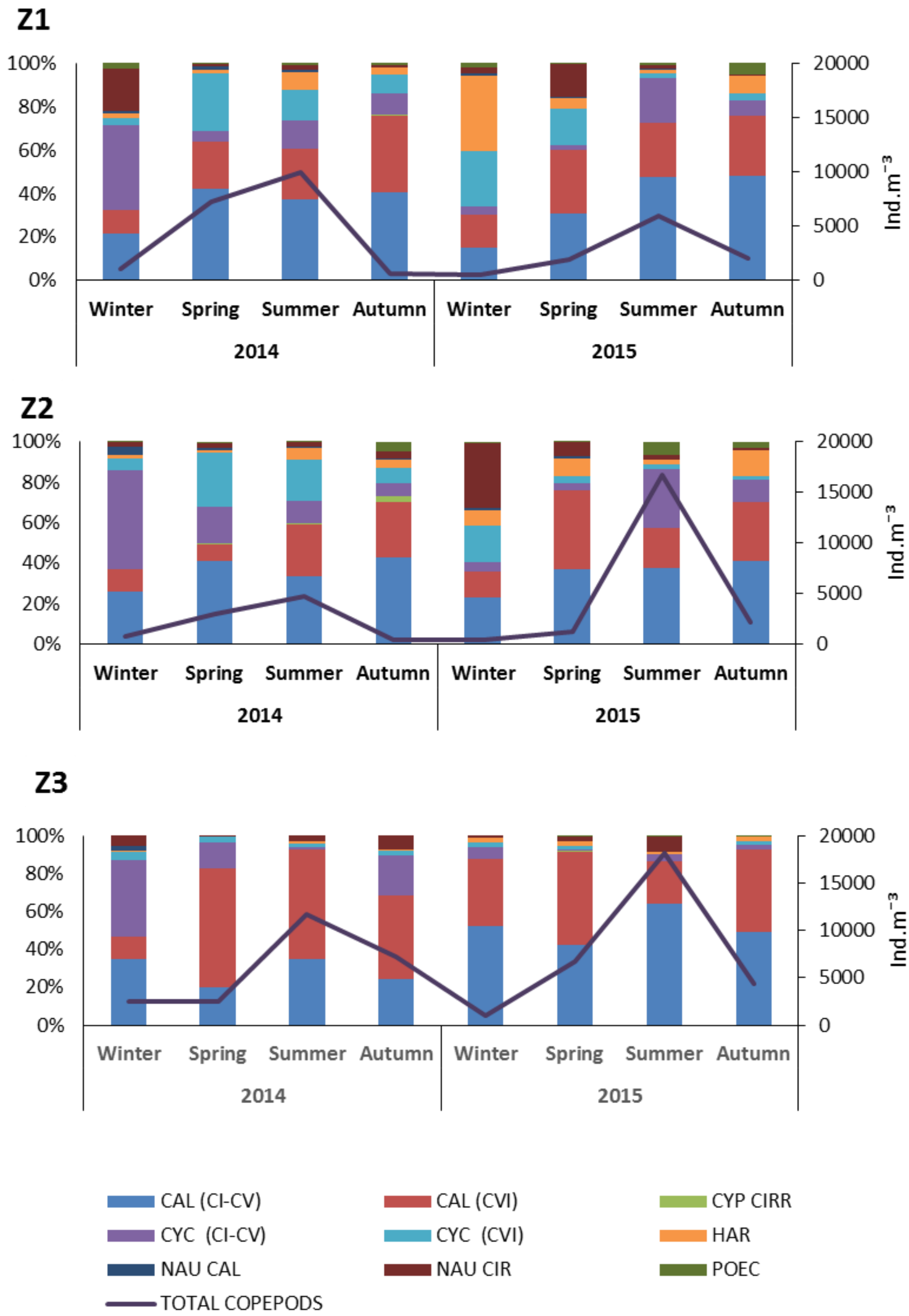

Figure 3.5: Abundance of the potential prey for *M. atlantica* and *M. kochii* at the three sampling zones. Relative percentage of the different taxa to the total abundance (bars). The line represents the total copepod abundance (Ind. m^{-3}). CAL (CI-CV): Calanoida (CI-CV); CAL (CVI): Calanoida (CVI); CYP CIRR: Cypris Cirripedia; CYC (CI-CV): Cyclopoida (CI-CV); CYC (CVI): Cyclopoida (CVI); HAR: Harpactacoida; NAU CAL: Nauplii Calanus; NAU CIR: Nauplii Cirripedia; POEC: Poecilostomatoida.

3.5.5 Relationships between biological and environmental factors

Table 3 shows the regression coefficients of the significant environmental variables and prey abundances correlated with *M. atlantica*, *M. kochii* nectophores and female and male gonophores abundances. In 2014, the model selected included salinity, calanoid nauplius and cirripedia cypris as important factors affecting nectophores of both *M. atlantica* and *M. kochii* and female and male gonophores. The positive regression coefficients denote positive influence of these factors on both species nectophores and on the gonophores. Thus, their abundance increases with increasing salinity and increasing abundance of calanoid nauplius and cirripedia cypris (Table 3.3).

In 2015, the best-fitted model selected included salinity and cyclopoida copepodites as significant factors affecting *M. atlantica* nectophores abundance (Table 3). On the other hand, the model selected for *M. kochii*, include only poecilostomatoida as a significant factor affecting their nectophores abundance, explaining 28% of the variation observed for this species (Table 3). For female and male gonophores, the best-fitted model included salinity and cyclopoida copepodites, respectively. (Table 3). As in 2014, the influence of the factors included in the models in 2015 was positive, denoting a concurrent increase of selected factors and *M. atlantica* and *M. kochii* nectophores and female and male gonophores (Table 3).

Salinity was the most significant factor explaining the abundance and distribution of polygastric and sexual stages during the both annual periods. The salinity regression coefficients were similar, yet slightly higher in 2015 (Table 3). Since the Zone factor was removed from the models due to collinearity with salinity, a significant influence of the latter also implies a significant influence of Zone. Thus, the distribution of *M. atlantica* and *M. kochii* nectophores and female and male gonophores showed an important spatial differentiation.

Table 3.3. Regression coefficients (β) from GLM analysis. PP: Precipitation; T: Temperature; Sal: Salinity; CAL (CI-CV): Calanoida (CI-CV); CAL (CVI): Calanoida (CVI); CYP CIRR: Cypris Cirripedia; CYC (CI-CV): Cyclopoida (CI-CV); CYC (CVI): Cyclopoida (CVI); HAR: Harpactacoida; NAU CAL: Nauplii Calanus; NAU CIR: Nauplii Cirripedia; POEC: Poecilostomatoida. *p<0.05, **p<0.01, ***p<0.001; (−) Excluded variables due to collinearity.

	M. atlantica (nectophores)		*M. kochii* (nectophores)		Gonophore Male		Gonophore Female	
	2014	2015	2014	2015	2014	2015	2014	2015
PP								
T								
Sal	0.01***	0.03**	0.02***			0.02**		0.02**
CAL (CI-CV)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
CAL (CVI)								
CYP CIR	0.28**		0.28**		0.23***		0.24***	
CYC (CI-CV)		0.35*						0.13*
CYC (CVI)								
HAR								
NAU CAL					0.15***		0.12***	
NAU CIR								
POEC				0.22***				
Zone	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
F-statistic	20.25	15.73	19.68	13.55	26.97	10.89	24.32	10.67
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001
R^2	0.52	0.48	0.51	0.28	0.60	0.24	0.57	0.37

3.6 Discussion

3.6.1 *Muggiaea* spp. and hydrology of the Mondego estuary

In this study we described the abundance and distribution annual patterns of *M. atlantica,* the most abundant gelatinous species in the Mondego estuary, and its congeneric *M. kochii,* identifying the environmental factors that affected the abundance and distribution of their both life cycle stages in the estuary.

The Mondego estuary showed a typical temperate seasonal pattern of precipitation, salinity and water temperature throughout studied period, in agreement with previous studies (e.g. Marques et al., 2006; Primo et al., 2012). Also, the salinity increase observed during 2015 was previously recorded on years with lower precipitation regime (e.g. Marques et al., 2014; Primo et al., 2011). The intrusion of marine waters in estuarine areas previously dominated by freshwater can strongly influence the hydrology and ecology of shallow estuaries like the Mondego (Marques et al., 2014). Also, different studies reported how changes in freshwater flow, due to drought events, promoted the entrance of marine species, and affected the structure and dynamics of the estuarine planktonic and fish communities (Baptista et al., 2010; Marques et al., 2014; Martinho et al., 2007; Primo et al., 2011).

Salinity, particularly in estuaries, significantly affects population size and asexual reproduction of cnidarians (Purcell, 2005), and may be considered as a proxy for physical processes (Blackett et al., 2014). The influence of salinity on *Muggiaea* species was particularly clear in our results, reflecting its influence on species abundance and distribution. Siphonophores are widespread in the oceans and most species are mainly referred as truly oceanic (Pugh, 1999). However, its presence has already been detected also in coastal areas, bays and estuarine waters (e.g. Molinero et al., 2005; Sanvicente-Añorve et al., 2009) and their higher vulnerability to currents and water mass movements (Lopez-Lopez et al., 2013; Palma and Silva, 2006) may result in higher transport inside the Mondego estuary, during low freshwater inflow periods, as in 2015. Salinity influence was clearer during the winter of 2015, when the unusual intrusion of saline waters allowed *M. atlantica* reproduction in Z1 and their flare through Z2. The exceptional conditions observed in 2015 affected also the spatial distribution of the species, allowing *M. atlantica* nectophores and *Muggiaea* spp. gonophores to reach upstream areas (Z3) of the estuary, increasing their abundance in these areas. Thus, the strong impact of salinity on the siphonophore community in the Mondego estuary seems related with both the transport of organisms from nearby coastal area and the spatial distribution of the species.

3.6.2 *Muggiea* spp. abundance and distribution

In recent years, *M. atlantica* has expanded its distribution range to the North Western of Mediterranean, suggesting that this species is able to exploit favourable environmental conditions more efficiently than other siphonophores (Licandro et al., 2012). Whereas, the first record in Atlantic Ocean of *M. kochii* was in 1924 in the English Channel when *M. atlantica* disappeared from the area, and its presence was an indicator of the inflow of water from the south (Angeles, 1971). In the Mondego estuary, *M. atlantica* has been recorded since 1994 (Azeiteiro et al., 1999), and its abundance has increased till the date of the present study. Conversely, *M. kochii* was considered a rare species until 2012, when its abundance started to rise and became a common gelatinous species in the estuary (D'Ambrosio et al., 2016). In the last decades, the massive proliferations of gelatinous organisms reported in some coastal areas and temperate seas (Attrill et al., 2007; Lynam et al., 2005) has been linked with significant changes in the functioning of marine and estuarine ecosystems (Condon et al., 2013; Lynam et al., 2004). It is worth noticing that the substantial abundance increases of these organisms can affect the population size of mesozooplankton, including fish eggs and larvae (Molinero et al., 2008; Sabatés et al., 2010), influencing fisheries either through top-down (predation) or bottom-up processes (competition) (Lynam et al., 2004; Purcell and Arai, 2001). Moreover, a number of recent studies have pointed out the influence of upwelling events in the distribution pattern of gelatinous zooplankton (Miglietta et al., 2008; Pavez et al., 2010; Sanvicente-Añorve et al., 2009) and how, in the Mondego estuary, coastal wind speed and direction exerted a direct effect on the structure and dynamics of gelatinous carnivore zooplankton community (D'Ambrosio et al., 2016).

The siphonophore community of the Mondego estuary showed a bimodal pattern of *M. atlantica* and *M. kochii* nectophores in 2014, with two peaks of abundance, June and October, concomitantly with an increase on female and male gonophores. Afterwards, during 2015, the mean seasonal abundance of these two species changed, displaying a unimodal seasonal peak, in summer. This seasonal variation on abundance of nectophores along with gonophores indicates optimal conditions for the sexual reproduction of the species. Blackett et al., (2014, 2015) also reported peak abundances during summer/autumn months, while in the Catalan Cost (Northwest Mediterranean), *M. atlantica* peaked in spring and early summer, and *M. kochii* in summer (Gili et al., 1987; Guerrero et al., 2016). Blackett et al. (2015) suggested a temperature critical basal limit of 10ºC for *M. atlantica* asexual reproductive activity in the English Channel. Additionally, our records of *M.*

atlantica nectophores and *Muggiaea spp*. gonophores in the Mondego estuary, during the winter of 2015 in the downstream zone (Z1), indicate that this species was able to sexually generate at water temperature near 13ºC.

During the studied period, *M. atlantica* and *M. kochii* showed a clear spatial pattern with the highest densities recorded in the downstream station (Z1), mainly influenced by marine water, and the lowest ones in the upper estuarine station (Z3), and markedly dominated by freshwater. This spatial pattern remained the same during both years, despite the superior abundances in 2015, mainly from *Muggiaea atlantica*. Previous studies have already demonstrated how, in a system such as Mondego estuary, gelatinous organisms are more abundant in the lower estuary (Marques et al., 2008; Pereira et al., 2014) and, according to Gili et al. (1987), *M. atlantica* and *M. kochii* commonly present the greatest density at the mouth of rivers, due to the their euryhaline and eurythermal characteristics.

In contrast with Blackett et al. (2015), who observed a close relationship between the abundance of the polygastric stage of *M. atlantica* and the *Muggiaea* spp. eudoxid stage, we cannot demonstrate which species produced more eudoxids in the estuary, due to their cohabitation with discrete high-amplitude peaks. However, in the winter of 2015, we may assume that gonophores found in Z1, were produced exclusively by *M. atlantica,* being the only species present at that time. This confirms the observations of Carré and Carré (1991), who showed that there was no production of eudoxids by *M. kochii* below 13ºC. Further, gonophores exhibited periods of absence before March-April, indicating that they matured slowly at lower winter temperatures while, in spring-summer, higher temperature might cause the fast release of eudoxids and a rapidly maturation of gonophores, increasing the abundance of *Muggiaea* spp. populations (Carré and Carré, 1991). The increased abundance of gonophores was concurrent with the increase in nectophores, showing higher abundances during summer months, at the downstream areas and during 2015. The increased abundance of nectophores and eudoxids (and consequently gonophores), is a clear sign of population growth, revealing optimal reproduction conditions (Purcell, 1982).

3.6.3 Prey influence on *Muggiaea* spp. distribution

The direct influences of local hydrology on *Muggiaea* spp. abundances may be extended by trophic interactions, since prey may also be affected. It has been shown that the distribution of siphonophores depends also on the food availability, being *M. atlantica* considered voracious predators (Greve, 1994; Mills, 1995; Purcell, 1982). Our results - increase of nectophores and gonophores – reinforce the optimal reproduction conditions during warmer months due to high availability of food. In 2014, both species and gonophores abundances were positively correlated with calanoid nauplius and cirripedia cypris. Other prey organisms than copepods are also important for siphonophores diet (Purcell, 1983, 1981), particularly during periods when copepods are scarce. In autumn 2014 was observed an increase of cirripedia cypris, and might be represent an important food source for both *Muggiaea* spp. peaks during a low copepod density period. According to Hansson et al., (2005), cirripedia are captured with high efficiency due to their extended appendages and lack of obvious escape responses, while copepodites can respond to hydrodynamic signals with pronounced escape behaviours (Kiørboe et al., 1999). In 2015, the positive relation of *M. atlantica* abundance with cyclopoida copepodites, and *M. kochii* nectophores with the copepod group of poecilostomatoida, reinforces the marine influence observed during this period, since that in the Mondego estuary, the cyclopoida species observed are mainly marine (Marques et al., 2006, 2014). Nevertheless, in this year there was an overall prevalence of marine prey species with significant changes mainly observed during winter and at upstream area Z3. However, this was not mirrored by the siphonophore abundance, probably because despite higher prey density, the prey composition, salinity and temperature was not ideal for the species growth. In 2015, as *M. atlantica*, female gonophores abundance was closely related with cyclopoida copepodites, indicating that this prey can be important for sexual reproduction of the species since the rates of eudoxid production and growth of *M. atlantica* increased with increasing density of prey (Purcell, 1982).

The peak of *M. atlantica* in 2015 seems to be related with both optimal prey availability and salinity values, and this combination could have been fundamental for the occurrence of the species bloom. Their accumulation may be favoured by reduced turbulence and vertical mixing associated with high stability of the water column (Graham et al., 2001; Licandro et al., 2012). These stable conditions allowed the salinity effect to be more pronounced. Despite the strong influence of salinity on *M. atlantica* in 2015, it seemed that salinity did not affect on *M. kochii* abundance in a great extent. The direct cause-effect of salinity on *M. kochii* seems less likely considering that this species is usually dominant in the relatively high saline waters of the southern Mediterranean and in different regions of the Atlantic Oceans (Martell-Hernández et al., 2014; Nagata et al., 2014; Zakaria, 2004).

This work described the different distribution of *M. atlantica* and *M. kochii* in Mondego estuary during two years, explained how salinity was the main factor that influenced their spatial and annual variation and how higher salinity values, as a consequence of lower precipitation and reduced river flow, has affected their seasonal abundance. Data on siphonophores are relatively scarce and the biology of these two species is still poor known. More studies are necessary to better understanding which variables most influenced their life cycle - adults and eudoxids stages - and their circulation in the system.

3.7 Acknowledgements

The authors are indebted to all the colleagues that assisted in the field and lab work. The present work was supported by FCT (Portuguese Foundation for Science and Technology) through a PhD grant awarded to Mariaelena D'Ambrosio (SFRH/BD/91541/2012), the postdoctoral grants attributed to SC Marques (SFRH/BPD/110400/2015) and AL Primo (SFRH/BPD/91030/2012), the Investigator FCT program attributed to F Martinho (IF/01410/2012), the COMPARE Project (FCOMP-01-0124-FEDER- 020850) financed by POPH (Portuguese Operational Human Potential Program), QREN Portugal (Portuguese National Strategic Reference Framework), and MCTES (Portuguese Ministry of Science, Technology, and Higher Education). E Guerrero gratefully acknowledge the Marine Biodiversity Conservation Group (MEDRECOVER) 2014SGR-1297.

CHAPTER 4

DIEL VARIATIONS IN THE FEEDING BEHAVIOR OF *MUGGIAEA ATLANTICA* IN THE MONDEGO ESTUARY

4.1 Abstract

In coastal ecosystems, calycophoran siphonophores *Muggiaea atlantica* have a significant predatory impact and a remarkable ecology role, being able, during its blooms, to remove a substantial proportion of planktonic prey, mostly small copepods, causing change in the structure and in the density of zooplankton communities. Since 1999, *M. atlantica* is the most abundant gelatinous predator in the Mondego estuary, a shallow system in the Iberian Peninsula, where its abundance has been increased during the last fifteen years. Thus, the aim of this study was to analyse the gastrozooids content of *M. atlantica* eudoxids caught over a half-tidal cycle, for estimating its predation rates and its prey selectivity. During the study, *M. atlantica* showed a similar abundance pattern of the gelatinous community, reaching the higher abundance value with the flood tide. In terms of prey, we considered several copepods groups for two different mesh size net (335 and 64 µm), as calanoids, cyclopods, harpacticods and, also, copepods nauplii. The majority of prey found inside gastrozooids were copepods nauplii, adults of *Euterpina acutifrons*, also carred of eggs and several copepods appendixes. Largely, greater variability of prey was found before the high tide, then, the variability decreased, until the low tide, when no full stomach was registered. *M. atlantica* eudoxids showed positive selection for *E. acutifrons* and negative selection for nauplii copepods, and their predation was estimated as 1.48 to 3.84 prey day $^{-1}$m^{-3}, equivalent to 0.16 µg C and 0.04 µg N assimilated.

Prey density significately affected the feeding selectivity of this species, and statistical analysis showed that the rise of salinity with the flood tide were the main physical factors that contributed to its increase in abundance. *M. atlantica,* due to the small size of their stomachs, only consumed the smaller sized copepods, as *E. acutifrons* and copepods nauplii, that represented, also, the most abundant zooplankton organisms presented in Mondego estuary.

4.2 Keywords

Gelatinous carnivore zooplankton**,** Siphonophorae, Calycophorae, Predation impact, Estuarine ecosystem

4.3 Introduction

Coastal zones and estuaries are important habitats that embrace a wide range of socioeconomic resources, i.e., food production, recreation and nutrient recycling, and are among the most valuable aquatic systems in terms of their services to human welfare (Costanza et al., 1997). In the last decades, some coastal areas have been subjected to massive long lasting proliferations of gelatinous carnivores (Attrill et al., 2007; Lucas et al., 2014), and this phenomenon has been linked with significant changes in the functioning of marine and estuarine ecosystems (Condon et al., 2011; Lynam et al., 2004). For instance, evidence has been shown how substantial increases of gelatinous abundance affect the population size of mesozooplankton, including fish eggs and larvae (Brotz et al., 2012) influencing fisheries either through top-down (predation) or bottom-up processes (competition) (Lynam et al., 2004; Purcell and Arai, 2001).

One of the major zooplankton carnivorous groups is constituted by siphonophores (Carré and Carré, 1991; Purcell et al., 2015), a class of pelagic and neritic free-swimming colonial species (Mapstone, 2014; Pugh, 1974). The siphonophore organisms are composed by "zooids", units genetically identical, but structurally different and functionally specialized for locomotion, feeding, defence, excretion, or reproduction of the colony (Dunn, 2005). In all siphonophores species, zooids are fixed in accurate species-specific arrangements, with the same sequence exactly repeated along the colony (Dunn et al., 2005).

4.3.1 Natural history

Muggiaea atlantica is a calycophoran siphonophore belonging to the neritic genera *Muggiaea* (Bush, 1851) (Mapstone, 2014). In recent years, *M. atlantica* has expanded its distribution range to the Northwestern of Mediterranean (Licandro et al., 2012) and in the Adriatic Sea (Batistić et al., 2007), has estabilished new population in Western English Channel (Blackett et al., 2014) and in the Gulf of Mexico (Martell-Hernández et al., 2014). Their life cycle comprises an asexual polygastric stage and a sexual eudoxid stage (Kirkpatrick and Pugh, 1984; Mackie et al., 1987); the eudoxids are free-swimming, with a stomach (gastrozooid), able to feed themselves and to release gonophores for several months that, via external fertilisation, generate a new polygastric colony (Carré and Carré, 1991; Mapstone, 2014).

In coastal ecosystems, *M. atlantica* has a significant predatory impact and a remarkable ecological role, being able, during its blooms, to remove a substantial proportion of planktonic prey, mostly small copepods (Purcell, 1981), causing change in the structure and in the density of zooplankton communities (Kršinić and Njire, 2001). Moreover, small siphonophores such as *M. atlantica,* when presented in high densities, are capable to pass through the mesh of the aquaculture cages, producing mass fish mortalities leading gill disorders (Baxter et al., 2011). Thus, studies of its feeding ecology are of high priority.

In Mondego estuary, a temperate shallow system in Iberian coast, the gelatinous community is mainly composed by hydromedusae and siphonophores, and *M. atlantica* is the most abundant gelatinous predator species (D'Ambrosio et al., 2016; Marques et al., 2006; Primo et al., 2012, 2009). In this ecosystem, the *Muggiaea* presence has been recorded since 1994 (Azeiteiro et al., 1999), and its abundance has increased till 2015, along with a salinity rise, due a very dry year (D'Ambrosio et al., submitted).

Considering the increasing number of gelatinous organisms recorded in coastal areas, the feeding activity and predation impact of different gelatinous predators have been already quantified, such as for *Aurelia aurita* in the Guadiana estuary (Pereira et al., 2014); for *Blackfordia virginica* in the Mira estuary; also for colonial organisms, as *Velella velella* in Liguarian Sea (Purcell et al., 2015). Moreover, Purcell has studied the dietary composition of epipelagic siphonophores, including *M. atlantica* (Purcell, 1982, 1981), but studies about its feeding behaviour are still scarce, especially on a short time-scale.

In the present study, we investigated the feeding activity and selectivity of *M. atlantica,* during a half semi-diurnal tide cycle (11-hours). Most of time, due to their fragility, when siphonophores colonies are captured by plankton nets, only the nectophores are caught as part of the colonies (Kirkpatrick and Pugh, 1984; Purcell, 1981). Thus, we analysed the gastrozooids content of *M. atlantica* eudoxids, which is the most direct approach to estimate, also, its predation rates and the digestion time of the different kind of prey (Purcell and Kremer, 1983). Furthermore, considering that assimilation is a vital process of energy transfer from one trophic level to the next (Conover, 1966), based on the prey ingested by *M. atlantica* eudoxids, we estimated the carbon and the nitrogen assimilated over the 11hour investigation.

4.4 Materials and methods

4.4.1 Study area

The Mondego estuary is a small temperate estuarine system on the western coast of Portugal (40°08’N, 8°50’W) (Fig. 4.1A), constituted by two arms, North and South, that join again near the mouth (Fig. 4.1B). The two arms are separated by the Murraceira Island, a large sediment island, and are characterized by different hydrological characteristics. The northern arm is deeper (4-8 m deep at high tide; residence time <1 day) and represented the principal navigation channel, being directly connected with the Mondego river. The southern arm is shallower (2-4 m deep at high tide; residence time < 3 days), more silted up in the upper zones, and the navigation mainly depends on the tides and on the freshwater input from the Pranto River, a small tributary of the Mondego, which discharges are controlled by a sluice (Cardoso et al., 2004; Marques et al., 2006).

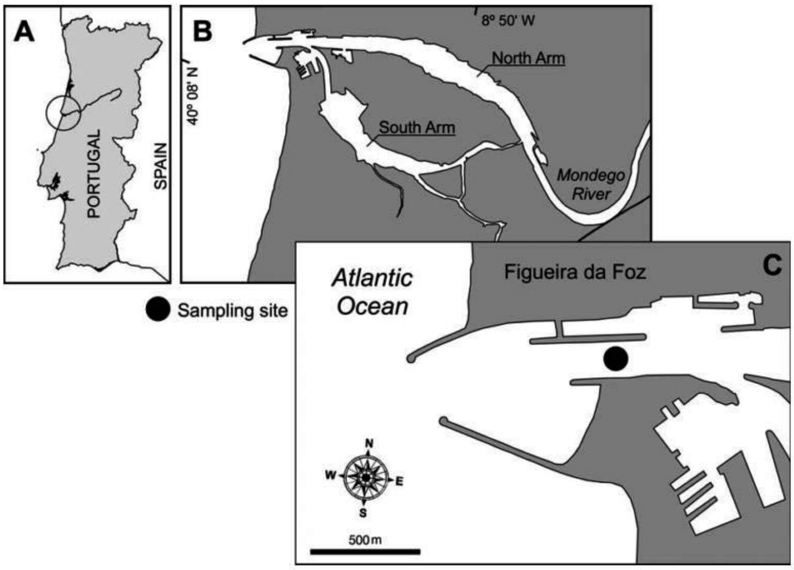

Figure 4.1. Map of the Mondego estuary and position of the sampling site in the estuary.

4.4.2 **Sample collection and laboratory work**

Samples were taken in a fixed station in the mouth of the estuary (Fig. 4.1C), where the influence of both river flow and neritic waters is strong. The sampling station was characterized by depths of 6 m at low tide and 13 m at high tide (Fig. 4.2A). *Muggiaea* organisms were collected on 24th September 2015, from 9.30 am to 7.30 pm, with a sampling frequency of one sample per hour. High and low tide were recorded at 12.30 am and 6.30 pm, respectively (Fig. 4.2A). Samples were collected by horizontal tows (10 cm below surface), using a plankton net (open diameter 0.5 m, mesh size 335 µm), and immediately fixed with 4 % buffered formalin. The volume of filtered water was estimated using a Hydro-Bios flow meter mounted in the opening of the net (the volume filtered per sample averaged 35±22 m^3).

In the case of *Muggiaea* species, they only develop a single anterior nectophore (Mackie et al., 1987), which provided a direct estimate of the polygastric (or asexual stage) abundance (Blackett et al., 2015). The free-living eudoxid is the sexual stage. The eudoxid comprises a bract that produces several males or females gonophores, and a gastrozooid, which is attached a tentacle, usually armoured with nematocysts, used to attract and capture the prey (Mackie et al., 1987; Mapstone, 2014).

In the laboratory, *M. atlantica* polygastric (asexual) and eudoxids (sexual) stages were separated under a dissecting microscope, identified and counted. Abundance of *M. atlantica* nectophores was expressed as individuals per m^3 (mean of two replicates ± standard deviation), whereas the abundance of the eudoxids gastrozooids was expressed as number of individuals per $m^{3.}$ For the feeding, the gastroozoids content of the eudoxids were identified under microscope "Zeiss Axiovert S100", and identification of the prey was made to species level whenever possible. The congeneric *Muggiaea kochii* has also been observed, however at very low abundances, while *M. atlantica* has been the most abundance over 13 years (D'Ambrosio et al., 2016). Even if the eudoxids are indistinguishable between the two species (Kirkpatrick and Pugh, 1984), on these basis, all the eudoxids found were considered produced by *M. atlantica* organisms.

Besides siphonophores, in order to estimate potential prey abundances, zooplankton samples were also collected using two plankton nets (open diameter 0.3 m, mesh size 64 µm; open diameter 0.5 m, mesh size 335 µm) and following the same sampling procedure as for siphonophores. Based on the literature (Purcell, 1983, 1981), we selected for the analysis the main taxa preyed by *M. atlantica,* which included different copepod groups (adults, copepodites and nauplii stage). For 64 µm net we chosen smaller organisms, such harpacticoda, copepodites of calanoida and cyclopoida and nauplii, whereas for the 335 µm net, we selected only calanoida and cyclpoida groups (adults and copepodites). The zooplankton analysis was carried out under a stereoscopic microscope and identification was made to group level. Abundance estimates were expressed as the number of individuals per m^3 (mean of two replicates ± standard deviation for the 335 µm net).

The surface environmental variables temperature and salinity were recorded *in situ* simultaneously at each zooplankton sampling using a conductometer (Cond 330i). Also, water sample was filtered for determination of chlorophyll a (Chl a), suspended and particulate organic matter (SPM and POM's).

4.4.3 **Data analysis**

During the study period, the feeding activity of *M. atlantica* eudoxids and the relative importance of each prey item was expressed, computing the following indices:

Occurance Index (OI), as a percentage of gastrozooids contained prey *i*; Numerical Index (NI), as a percentage of prey *i* in the gastrozooids; Vacuity index (VI) as a percentage of empty gastrozooids (Hyslop, 1980). Moreover, to describe the *M. atlantica* preference for prey and the feeding activity of the eudoxids, the Jacobs Index (D) were calculated, according to the formula: $D = (r - e)/(r + e – 2re)$, where r and e are the relative frequency of prey i in the gastrozooids and in the environment, respectively. D value is between -1 (negative selection) and 1 (strong preference), values close to zero mean that the prey is used in proportion to its availability (Jacobs, 1974). Additionally, predation rates per eudoxids were calculated for the two prey types found intact in the gastrozooids of the eudoxids through the 11-hour sampling, i.e. harpacticoida (≤0.35mm copoepods) and copepods nauplii, by summing the results of the following equation:

Σ (% of prey (av. nº prey eudoxid^{-1})11h/digestion time), (Purcell, 1982).

The digestion time of the prey was estimated based on the results of Purcell (1982), which suggested a maximum digestion time between 2 and 4 hours at ambient temperature of 8 to 10ºC. In our study, we calculated the digestion time for each prey group using an average temperature of 18ºC, for the sake of simplicity, we considered a linear relationship between temperature and digestion time, although this relationship can follow a non-linear behaviour (Ruel and Ayres, 1999).

Carbon (C) and nitrogen (N) ingestion by eudoxids during the sampling period were calculated, estimating the dry weights of the prey according to regression equations determined by Durbin and Durbin (1978), wich related length to dry weight in *Acartia clausi*. Weight was estimated to be 30% low and, following Purcell (1982), in the present study a correction of 30% was used. For harpacticoida was used the regression: weight= 9.63 (cephalotorax length)^3.059, where cephalotorax length= 0.6mm (Purcell, 1982); for nauplii weight= 19.04 (total length)^2.849, where total length= 0.0327 mm (Durbin and Durbin, 1978). Dry weight was converted to C and N by multiplying by 46.04% C and 11.3% N measured for the harpacticoid *E. acutifrons* in Ara (2001) and multiplying by 11.38% C and 1.84% N for nauplii, measured for *A. tonsa* copepodites in Jones et al. (2002).

Moreover, to test the relationship between *M. atlantica* and physical environmental factors, Multiple Regression Analysis and Principal Component

Analysis (PCA) on the 11-hours abundance of *M. atlantica* were performed in Statistica 7. The interpretation of the first (PC1) and second (PC2) principal components, which together accounted for 91, 80% of the total variance, involved five environmental descriptors (water temperature, salinity, POM's, SPM and Chla) and *M. atlantica* nectophores as supplementary variable.

4.5 Results

4.5.1 Environmental drivers

In figure 2 are shown the values of the environmental parameters recorded during the study. The water temperature (Fig. 4.2b) showed a typical dial pattern, with representative values of late summer in the Mondego estuary. The minimum value was recorded early morning (15.5ºC) and the maximum at midday (19.8ºC), with a continuous decline of the temperature until evening (17.4ºC at 7.30 pm). Generally, salinity (Fig. 4.2b) recorded an increase until the maximum tidal amplitude, peaking early morning (34.5), then salinity decreased, reaching lower values late afternoon (27.6).

Fluctuations of SPM and POM's values over the tidal cycle were observed (Fig. 4.2c). During early morning, high values of SPM and POM's were measured (54.6 mg/l and 9.5 mg/l, respectively), then, with the rise of the tide, suffered a decline (35.3 mg/l and 4.5 mg/l, respectively). Chlorophyll a showed a similar pattern of SPM and POM's (Fig. 2d), reaching higher values on early morning (2.8 mg/m^3), decreasing with the rise of the tide (0.6 mg/m^3) and growing again as the tide dropped. Table 1 shows the maximum and minimum values of the environmental factors.

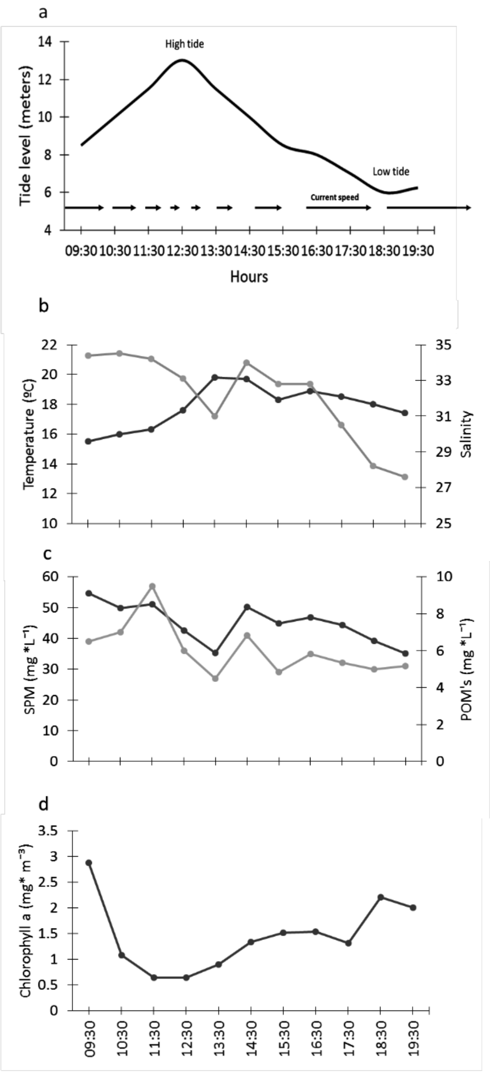

Figure 4.2: a: Tidal scheme at the sampling point during the study. Arrows denote the current speed; b: Temperature (black line) and salinity (grey line); c: Suspended (SPM) (black line) and organic (POM's) (grey line) particulate matter (mg L^{-1}); d: Chlorophyll a (mg m^{-3}) recorded during samplig period.

Table 4.1: Maximum and minimum values (±SD) of environmental factors recorded during the studied period.

Env. Param.	**Max ± SD**	**Min±SD**
Temperature (ºC)	19.8±1.14	15.5±1.14
Salinity	34.5±0.02	27.6±0.02
SPM (mg/L)	54.6±5.09	35.3±5.09
POM's (mg/L)	9.5±1.02	4.5±1.02
Chl a (mg/m³)	2.8±0.51	0.6±0.51

4.5.2 Biological data

4.5.2.1 *Muggiaea atlantica* and gelatinous community

In table 4.2 is displayed the list of the gelatinous carnivores' species presented during the sampling period in Mondego estuary (total abundance±SD and total percentage), mainly represented by hydromedusae (11 species) and siphonophores (2 species).

Table 4.2: Total abundance (ind m^{-3}±SD) and total percentage of the gelatinous carnivore species presented during the sampling period in Mondego estuary.

Species	**Total Abundance ±SD** (ind m^{-3})	**%**
M. atlantica	79.46±19.52	58.23
Obelia sp.	20.47±11.54	15.00
Lizzia blondina	11.74±9.86	8.61
Clytia hemisphaerica	8.83±7.19	6.47
N. I. Species	8.64±4.02	6.33
Bougainvillia muscus	3.33±3.29	2.44
M. kochii	2.57±2.87	1.88
Ctenophora sp.	0.44±0.18	0.32
Podocorynoides minima	0.41±0.34	0.30
Sarsia sp.	0.19±0.06	0.14
Rathkea octopunctata	0.17±0.20	0.13
Margelopsis haeckeli	0.10±0.06	0.07
Liriope tetraphylla	0.07±0.06	0.05
Sarsia tubulosa	0.03±0.00	0.02

Muggiaea atlantica was the most abundant species 2 hours after the peak tide, comparing to the total abundance of the gelatinous carnivores (Fig. 4.3), showing the highest polygastric abundance in the first hour of sampling (14±1.1 ind m^{-3}). *M. atlantica* and gelatinous community showed a similar abundance pattern, their abundance decreased during the high tide (*M. atlantica* abundance: 3.8±1.1 ^{33}; community abundance: 1±0.6 Ind m^{-3}) and, while the tide dropped, the *M. atlantica* nectophores abundance increased again, whereas the gelatinous community showed the highest abundances value of the study (14.8±14.8 Ind m^{-3}). Then, with the falling of the tide, *M. atlantica* and gelatinous community reached the lowest abundance values (0.5±0 for *M. atlantica* and 0.96±0 Ind m^{-3} for the community).

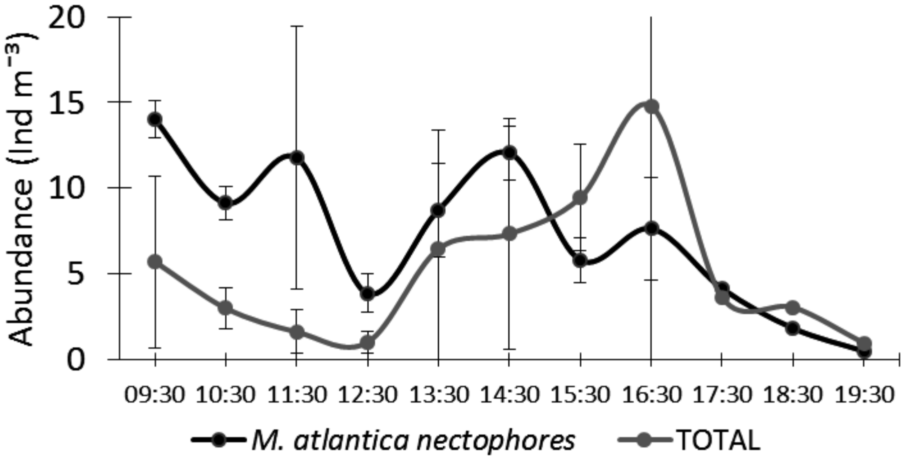

Figure 4.3: *Muggiaea atlantica* abundance and total abundance of the gelatinous carnivores' species identified during the study in Mondego estuary.

The multiple regression analysis (Table 4.3) revealed that the abundance of *M. atlantica* significantly and positively increased with salinity (S) as well as POM's and Chl a, being expressed by the equation: M= 536.45+0.70 S+0.65 POM's+0.38 Chl a (r^2=0.95, Table 4.3), which was also, confirmed by the Principal Component Analysis (PCA) (Fig. 4.4). There were no significant difference of *M. atlantica* nectophores and the total gelatinous carnivores' abundance between high and low tide (ANOVA, p>0.05).

Table 4.3: Multiple regression analysis between *M. atlantica* and environmental variables. Regression equations are given in text.

Variables	**Coefficient**	**t**	**p**	**r^2**
Intercept	536.45	3.23	0.02	0.95
Salinity	0.70	3.02	0.03	
POM's	0.65	3.56	0.02	
Chl a	0.38	2.93	0.03	

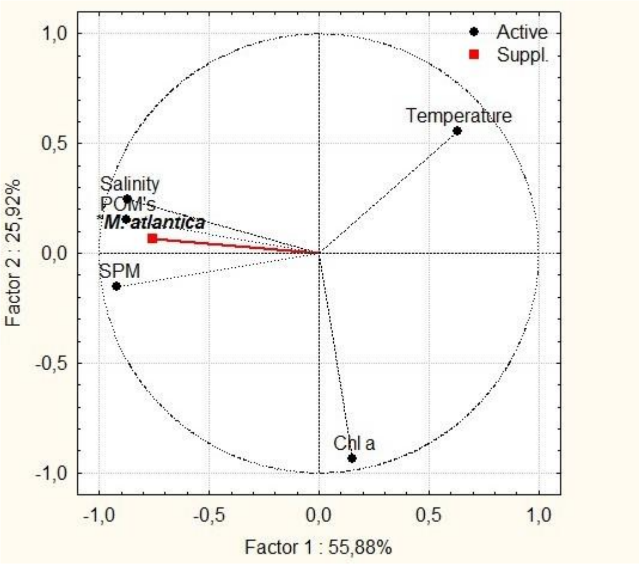

Fig 4.4: Results of the principal component analysis (PCA) performed on abundance of *Muggiaea atlantica* nectophores and the environmental factors (temperature, salinity, POM's, SPM and Chl a).

4.5.2.2 Prey abundance

Table 4.4 and figure 4.5 show the tidal variability and total abundance of the *Muggiaea atlantica* potential prey caught with two different mesh size nets. For larger mesh size net (335 µm), calanoida were constantly the most abundant groups (98% of the total prey abundance) (Table 4.3; Fig. 4.5a), reached the minimum during the high tide (9.19±7.7 Ind m^{-3}), and peaked when the tide decreased (363±60.8 Ind m^{-3}). Compared to calanoida, cyclopoida abundance was lower (2% of the total prey abundance) (Table 4.4, Fig. 4.5a), reaching the maximum value during the low tide (6.63±0 Ind m^{-3}) and the minimum during high tide (0.08±0 Ind m^{-3}).

Table 4.4: Total abundance (Ind m^{-3}±SD) and total percentage of different *Muggiaea atlantica* potential prey caught with 335µm and 64 µm mesh size net.

Species	**Total Abundance ±SD** (Ind m^{-3})	**%**
335µm		
Calanoida (±SD)	918.96±267.75	97.93
Cyclopoida (±SD)	19.34±4.28	2.06
64µm		
Calanoida	21696	1.99
Nauplii Copepods	665024	61.03
Cyclopoida	57664	5.29
Harparcticoida	345200	31.68

Regarding the smaller mesh size net (64 µm), the most abundant prey groups were represented by nauplii copepods and harpaticoida (61% and 32% of total prey abundance, respectively) (Table 4.4), both groups showed higher abundance during high tide (300.000 Ind m^{-3} and 134.144 Ind m^{-3} for nauplii and harpacticoida, respectively), and lower during low tide (3300 Ind m^{-3} and 2688 Ind m^{-3} for nauplii and harpacticoida, respectively) (Fig. 4.5b). There were no significant differences of prey abundances between high and low tide (ANOVA, $p>0.05$). Calanoida group included mostly *Acartia tonsa*, then *Acartia clausi* and *Calanus* spp.; cyclopoida group included mostly *Oithona* spp.; harpacticoida group completely represented by *Euterpina acutifrons* (author note).

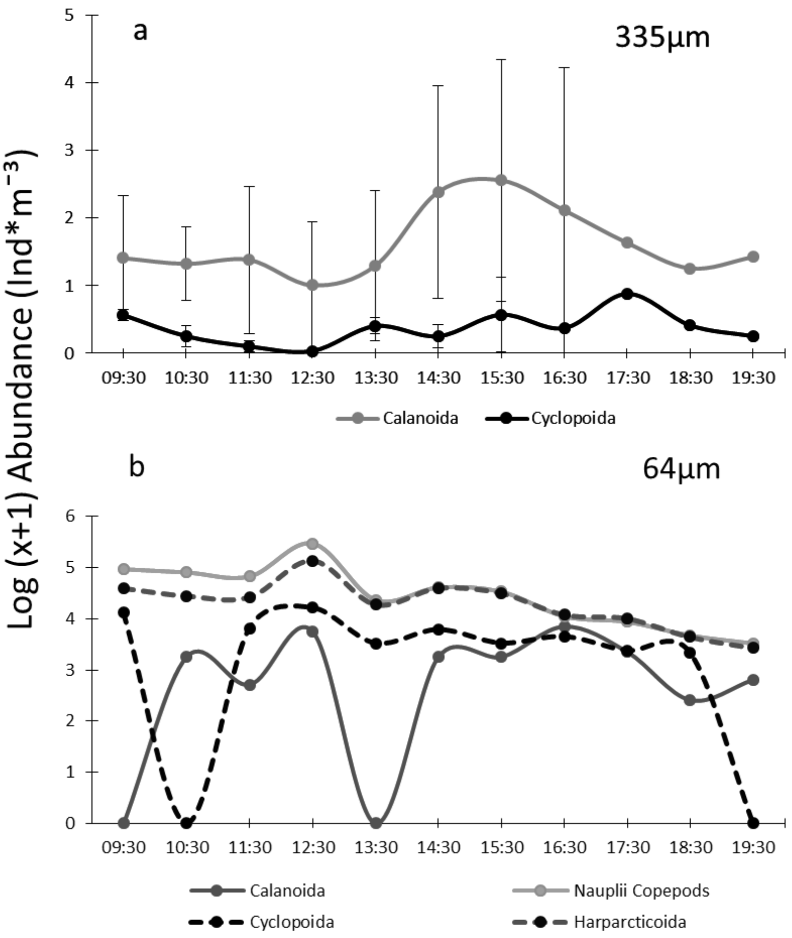

Figure 4.5: Abundance of the potential prey for *M. atlantica*. a: prey caught with 335 µm mesh size net; b: prey caught with 64 µm mesh size net. To better appreciate the groups distribution, data were log-transformed.

4.5.2.3 Feeding, predation rates, C and N assimilation

Figure 4.6 shows the results concerning feeding selectivity and stomach content of *M. atlantica* eudoxids during the sampling hours. The number of the eudoxids with gastrozooids was highest when the tide grew (210 eudoxids with gastrozooids at 9.30 am), gradually reducing until the tide peak, when it rose again (84 at 1.30 pm). Then, their number increased again, peaking before low tide (87 at 4.30 pm). During the low tide, the lowest number of eudoxids with gastrozooids was recorded (2 at 7.30 pm) (Fig 4.6 a, 4.6b). The number of full stomachs showed a similar pattern of the gastrozooids, the higher number were found while the tide grew (10 full stomachs at 9.30 and 11.30 am, corresponded to 4% and 11.3% of the total, respectively). Then the number decreased, peaking again before the low tide (13 full stomachs at 4.30 am, corresponding to 15, 3%). Overall, the vacuity index was high (92.91; table 4.5) and, during the last three hours of sampling, no full stomach was found.

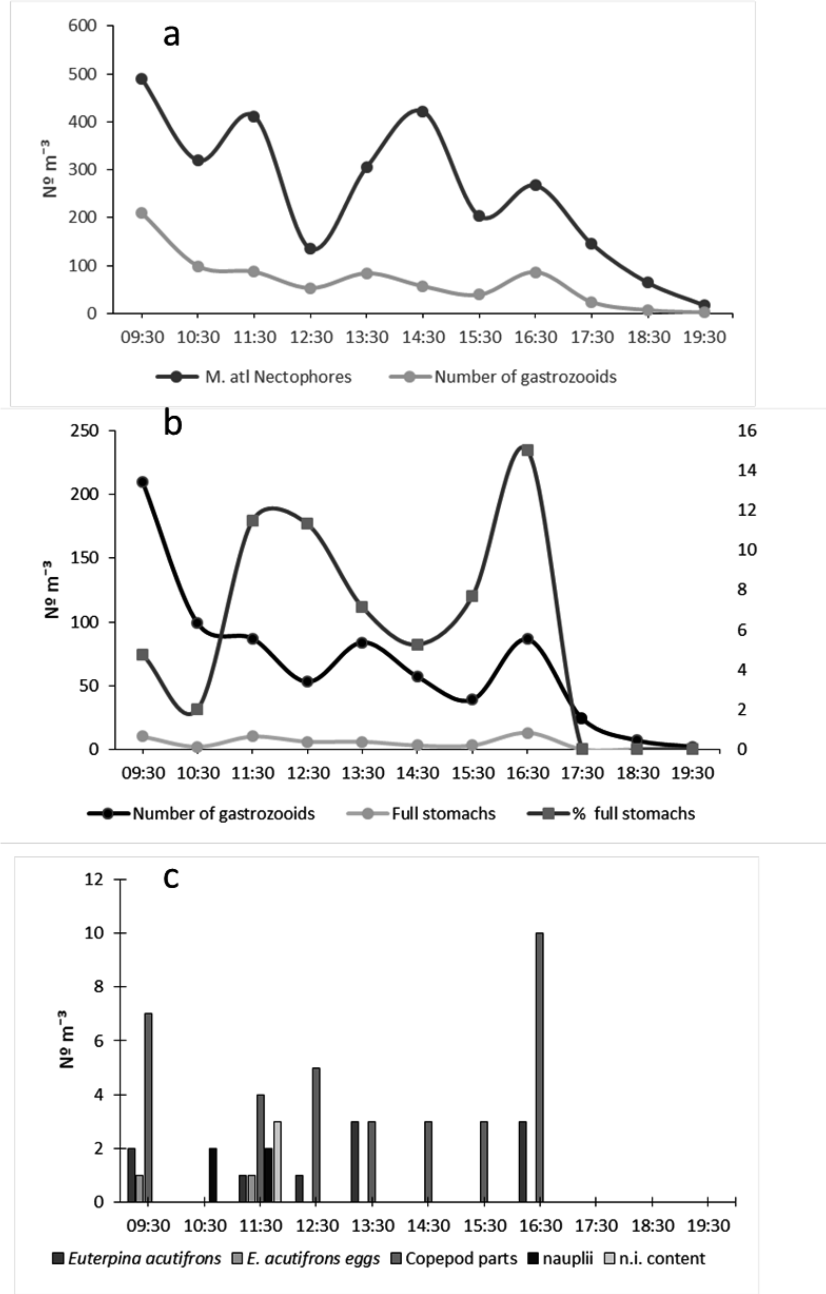

Figure 4.6: Feeding selectivity and stomach content of *M. atlantica* eudoxids during the different sampling hours in Mondego estuary. a: number of *M. atlantica* nectophores and eudoxids with gastrozooids; b: eudoxids gastrozooids and full stomachs of *M. atlantica*; c: stomach content of eudoxids of *M. atlantica.*

Regarding stomach content of the *M. atlantica* eudoxids (Fig 4.7a), generally were found adults of *Euterpina acutifrons* (Table 4.5 and Fig. 4.7b), also carred of eggs, copepods nauplii integers (Fig. 4.7c), several copepods appendixes (Fig. 4.7d), and unidentified stomach contents, probably because were already in the digestion phase (Fig. 4.6c). Largely, greater variability of prey was found before the high tide (Fig. 4.6c), when *Euterpina acutifrons* with eggs, copepods nauplii, several copepods appendixes were found. Then, the variability decreased, until the low tide, when no full stomach was found. Jacobs selectivity index for the two prey identified in the gastrozooids, showed positive selection for *E. acutifrons* and negative selection for copepods nauplii (Table 4.5).

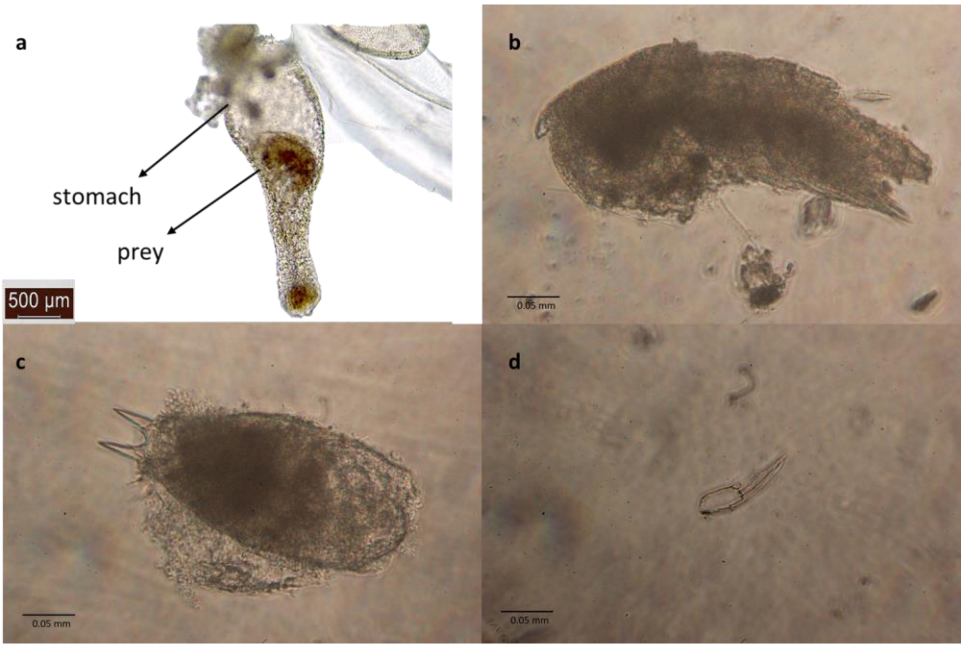

Figure 4.7: Examples of the gastrozooids content of the *M. atlantica* eudoxids. a: eudoxid gastrozooid with prey; b: *Euterpina acutifrons*; c: copepods nauplii; d: appendix. (Photographs by Joana Cruz).

Table 4.5: Prey occurrence index (OI), numerical index (NI), Jacobs selectivity index (D) calculated based on the two prey identified in the gastrozooids and Vacuity index (VI) for the eudoxids gastrozooids during the period investigated.

Prey	**OI**	**NI**	**D**	**VI**
Euterpina acutifrons	18.86	18.86	0.69	
Nauplii copepods	7.54	7.54	-0.59	
				92.91

During the 11-hours study, considering predation rates, an average of 0.16-0.18 nauplii and 0.20-0.72 *E. acutifrons* were captured by each eudoxid, wich totalled 0.35-0.90 prey eudoxid^{-1}sampling period $^{-1}$. Eudoxids density averaged 2/m^3, so 0.68-1.76 prey sampling period $^{-1}$m^{-3} were consumed by *M. atlantica* eudoxids, equivalent to 1.48 to 3.84 prey day $^{-1}$m^{-3}. Thus, the dry weight of prey egested by *M. atlantica* eudoxids was estimated to be 0.156 µg/eudoxid/11hours, which is equivalent to 0.072 µg C and 0.018 µg N assimilated, or 0.16 µg C and 0.04 µg N por day.

4.6. Discussion

4.6.1 Environmental factors

This work examined the distribution and the predation impact on the zooplankton community and the feeding selectivity of *M. atlantica*, the most abundant gelatinous carnivore in Mondego estuary, during a half-tidal cycle. Through our study, its abundance was higher with the rising tide and decreased while the tide dropped. Furthermore, the sea water input into the estuary increased significantly with the rise of the tide, reflecting the increase of salinity until the maximum tidal amplitude. In an estuary, water circulation is mostly driven by the tidal excursion, and the seawater influence reflected by the increase of salinity in the water (Liu and Liu, 2014), is expected to increase during higher amplitude tides, promoting structural changes in the zooplankton estuarine community (Marques et al., 2006). Tides in the Mondego estuary are semi-diurnal, with a median daily tidal range of 3.5 meters, and a full tidal period covering about 12.4 hours (Duarte et al., 2002). The dynamics of estuarine communities are influenced by the interactions of both physical and biological processes (Kimmerer, 2004), and at shorter time scale, physical effect, e.g. variation in freshwater flow, tidal moving and salinity distribution, tend to dominate (Kimmerer, 2004). In the Mondego estuary at daily scale, as our study described, the higher vulnerability to currents and mass movements (Marques et al., 2006) and the rise of salinity with the flood tide, as Pearson correlation confirmed, were the main physical factors that contributed to the increase of *M. atlantica*. In long-term scale, salinity, particularly in estuaries, significantly affects population size and asexual reproduction of cnidarians (Purcell,

2005) and, in the Mondego estuary, salinity was found as one of the main drivers who affected the abundance and distribution of *Muggiaea* spp. in the period 2003-2010 (Primo et al., 2012) and in 2015 (D'Ambrosio et al., submitted).

4.6.2 *Muggiaea atlantica* and gelatinous carnivore community in the Mondego estuary

Since 1994, the presence of *M. atlantica* in the Mondego estuary has been constant (Azeiteiro et al., 1999), with seasonal abundance peaks in spring and summer, becoming the most abundant gelatinous carnivores species during the decade 2003-2013 (D'Ambrosio et al., 2016), and reached the maximum abundance value in 2015, when higher salinity values, as a consequence of lower precipitation and reduced river flow, have affected their seasonal abundance (D'Ambrosio et al., submitted).

In our study, the gelatinous community showed a similar abundance pattern of *M. atlantica*, reached the higher abundance value with the flood tide. Besides siphonophores, *Lizzia blondina* and *Obelia* spp., since 2003, are the most abundant hydrozoan species in Mondego estuary (D'Ambrosio et al., 2016; Marques et al., 2006) and, also, mainly represented the gelatinous carnivores community at high tide during 2003-2004 (Marques et al., 2006). In small estuaries like Mondego, gelatinous carnivore organisms may take advantage of tides to enter the estuary and their accumulation may be favoured by reduced turbulence and vertical mixing associated with high stability of the water column (Condon et al., 2012; Licandro et al., 2012; Primo et al., 2012). Here, through predation and competitive interactions with planktivorous fish (Kaneda et al., 2007), gelatinous organisms have the crucial role in moulding estuarine and coastal zooplankton communities (Guerrero et al., 2016; Pereira et al., 2014). Indeed, when they occur in large number, prey population can be significantly affected (Purcell, 1982; Sabatés et al., 2010), as happened in the Adriatic Sea in 1997, when an invasion of *M. atlantica* caused changes in the structure, distribution and density of nauplii, copepodids and adult small copepods (Kršinić and Njire, 2001), and this predation indirectly controls the population size of other zooplankton organisms, such as fish larvae (Hansson et al., 2005).

4.6.3 Prey density

Several studies have already shown the role of *M. atlantica* and gelatinous carnivores as a voracious planktivorous predator and potential competitors of fish (Batistić et al., 2013; Purcell, 1981; Purcell and Arai, 2001). Their growth rate tends to increase with prey density, such as crustacean development stages and small copepods (Purcell, 1982), the major constituent of zooplankton community in Mondego estuary (Marques et al., 2006).

In this study, for the larger mesh size net, calanoids made up 98% the total of copepod catch and the remain 2% was represented by cyclopoids, and their abundance was lower during high tide, in accordance with highest densities of *M. atlantica* and the gelatinous carnivores community (Purcell, 2005, 1982). This gelatinous carnivores-copepod link was previously reported by Primo et al. (2012), which revealed significant relationship between summer gelatinous zooplankton community and the copepod *Acartia clausi,* the major constituent of the mesozooplankton community in Mondego ecosystem. Conversely, the daily pattern of the harparcticoids and copepods nauplii, caught with the smaller mesh size net, was consistent with gelatinous zooplankton abundance. The small-sized copepods (<1mm) (Turner, 2004), such as belonging to the harpacticoid group, in this study entirely represented by the species *Euterpina acutifrons*, are important links in marine food webs, serving as major grazers of phytoplankton and as prey for ichthyoplankton and other larger pelagic carnivores, such gelatinous carnivores (Gordina et al., 2005; Turner, 2004).

4.6.4 Feeding and predation rates of *M. atlantica*

In the present study, the higher number of full gastrozooids of *M. atlantica* eudoxids was found when the tide grew (11%) and before low tide (15%), corresponded to the higher abundance of *M. atlantica* nectophores. The majority of eudoxids found have empty gastrozooids, as represented by the high value of vacuity index (92.91%). This index represents an inverse indication of feeding intensity, and its value was so high probably due to the prey ejection when organisms are preserved in formalin-water, or may indicate non-continuous feeding, different feeding rates throughout the day or rapid prey digestion (Marques et al., 2015). Purcell in two different studies (1981, 1982), compared the feeding pattern of *M. atlantica* between day and night, and observed that the feeding was higher at night. Our results showed higher percentage than those presented in these studies during day time, and no full gastrozooids were found in the evening, although we considered only gastrozooids of eudoxids and not of adult colonies, as Purcell as described in most studies on feeding behaviour of siphonophores (Purcell, 1983; Purcell and Kremer, 1983).

Gastrozooids content analysis of *M. atlantica* eudoxids, revealed low variability in prey selection, since only two different types of prey in the stomachs have been identified, copepod nauplii and the harpaticoid copepod *Euterpina acutifrons,* as well as several appendices of copepods. Based on the results of the Jacob index, *M. atlantica* showed positive selectivity for *E. acutifrons* and negative selectivity for copepods nauplii. Prey selectivity can vary between time of the year and areas and, also, is highly dependent on the structure of the zooplankton community (Purcell, 1992). In the present study, these prey represented the most abundant and smaller zooplankton groups in Mondego estuary and, being gelatinous non-visual predators (Purcell, 1981), their selectivity in terms of the prey, depends on their density in the environment (Marques et al., 2015). Moreover, our results are in agreement with Purcell (1981, 1982, and 1983) that described how the calycophorae *M. atlantica*, due to the small size of their stomachs, only consumed the smaller sized copepods, including all stages of small calanoid copepods, and harpacticoid copepods. Food items found in the gastrozooids have been listed for different species of siphonophores. Purcell (1983) identified 80-100% of the stomach content of the siphonophores *Forskalia edwardsi*, *F. tholoides*, *Rosacea cymbiformis*, *R. flaccida* and *Diphyes dispar,* was composed by copepods, and the same results were found for the siphonophore *Sphaeronectes gracilis* (Purcell and Kremer, 1983), while siphonophores with larger stomachs, e.g. suborders Cystonectae or Physonectae, fed also non-copepod prey, such fish larvae, shrimp, ostracods. In situ studies have enabled us to discover that many siphonophore species are highly selective in their feeding, with the type of prey captured depending on certain morphological and behavioural characteristics of the individual species. These characteristics include the size and number of gastrozooids and tentacles borne on the stem; the types of nematocysts present; and the specific fishing behaviour (Pugh, 1989).

During the investigated period, *M. atlantica* eudoxids were estimated to consume 0.68-1.76 prey sampling period $^{-1}$m^{-3}, equivalent to 1.48 to 3.84 prey day $^{-1}$m^{-3}, which certainly underestimated results as the night-time predation was not considered. These values of prey consuption were lower compared with the results of Purcell (1982), who estimated a consumption of 7.5-14.4 prey day^{-1}m^{-3} for polygastric colonies of *M. atlantica,* probably because we only considered prey consumed by eudoxids. The efficiency of prey consumption of these organisms depends by their digestion rates. This process is correlated with their respiration rates and their metabolic processes, and siphonophores with higher respiration rates digested prey more rapidly (Biggs, 1977; Purcell and Kremer, 1983). At a water temperature of 21º-22ºC, calycophoran *Diphyes dispar*, digested prey in 5.8 hours, displayed intermediate digestion rates values than other siphonophore groups, which varied between 1.6 and 9.6 hours (Purcell, 1983). Regarding other siphonophore groups, calycophores siphonophores are slower swimmers, but still capable of rapid swimming movements for escape and spreading the tentacles for feeding, and the range of respiration and excretion rates in this group was correspondingly broad (Biggs, 1977; Purcell and Kremer, 1983). These results provide useful insights to understanding the biology of *M. atlantica*, including the first data on the dial feeding behaviour of its eudoxids and estimated assumption of the two prey types of this important coastal predator species. Moreover, we observed a diet selectivity by *M. atlantica* eudoxids, probably because the smaller sizes of *E. acutifrons* and copepods nauplii, and because their represented the highest abundances in the zooplankton community in Mondego estuary. The progressive expansion of the distribution of *M. atlantica* into some regions (Batistić et al., 2013; Blackett et al., 2014) suggest that this species can take advantage of favourable environmental conditions more efficiently than other siphonophores (Licandro et al., 2012), with direct and indirect influences on the trophic chain. The increase of salinity caused by the flood tide was the main physical driver that influenced the entrance of *M. atlantica* in the Mondego estuary, and has a remarkable influence on the abundance of this species.

4.7 Acknowledgements

The authors are indebted to all the colleagues that assisted in the field and lab work. The present work was supported by FCT (Portuguese Foundation for Science and Technology) through a PhD grant awarded to Mariaelena D'Ambrosio (SFRH/BD/91541/2012), the postdoctoral grants attributed to SC Marques (SFRH/BPD/110400/2015), the Investigator FCT program attributed to F Martinho (IF/01410/2012), the COMPARE Project (FCOMP-01-0124-FEDER- 020850) financed by POPH (Portuguese Operational Human Potential Program), QREN Portugal (Portuguese National Strategic Reference Framework), and MCTES (Portuguese Ministry of Science, Technology, and Higher Education). E Guerrero gratefully acknowledge the Marine Biodiversity Conservation Group (MEDRECOVER) 2014SGR-1297. J Cruz gratefully acknowledge the European Regional Development Fund (COMPETE, Program Operational Competitiveness Programme) and FCT national fund (IP-PTDC/MAR-BIO/0440/2014).

CHAPTER 5

GENERAL DISCUSSION

5.1 Gelatinous zooplankton in a shallow estuarine system (Mondego estuary)

As the Anthropocene advances, certain species are thriving as the human machine decimates others (McCauley et al., 2015). The ocean faces a “gelatinous future” dominated by jellyfish, which have managed to survive ice ages, warming climates, all five mass extinctions and even humans, and will prosper in the ecological niches unoccupied by fish (Condon et al., 2013).

Multiple explanations have been suggested to account for the increased frequency and intensity at which jellyfish blooms are being observed (Doyle et al., 2008; Lynam et al., 2005; Molinero et al., 2008; Primo, 2012). Gelatinous have the ability to reproduce rapidly through asexual reproduction and further show extremely high prey consumption rates, which enable them to exert control over energy flow and nutrients, thereby causing changes in conventional fish dominated food webs (Lynam et al., 2006). In addition, their opportunistic life history traits, including high fecundity, rapid growth rates and short generation times, enable them to respond to suitable conditions with dramatic localized population increase, a “true” bloom (Graham et al., 2001).

The importance of gelatinous zooplankton community in estuaries and coastal environment (Graham et al., 2001; Purcell, 2012), highlight the need for a clear understanding of their seasonal dynamics and abundance, and that was one major aim for the present work.

During the studies of this dissertation, based on a dataset comprising a decade (2003-2012), gelatinous community in the Mondego estuary was represented by 20 different taxa of Hydromedusae and 2 Calycophora siphonophores species, *Muggiaea atlantica* and *M. kochii*. Since 2003, the dominant hydrozoans were the species *Lizzia blondina* (Anthomedusae), *Obelia* sp. (Leptomedusae) and *Liriope tetraphylla* (Trachymedusae), mainly in the summer, exhibiting maximum densities in the lower reaches of the estuary and at high tide. These species are, also, common in NW Mediterranean Sea, were *L. blondina* peacks in June and September

(Gili et al., 1988; Sabatés et al., 2010), following the same pattern as in the Mondego estuary, or in the South Adriatic Sea, were *Obelia* sp. formed blooms. Instead, *Liriope tetraphylla* was rarely recorded in the Adriatic Sea (Pestorić et al., 2012), while its blooms in the summer of 2006 and 2007 in the Sea of Marmara, caused mucilage events and a shift in the zooplankton community structure (Yilmaz, 2015). In the Mondego estuary, generally, two main peaks of abundance have been described, one in April-May, and the other one in July-August, dominated mainly by siphonophores. Largely, the maximum abundance of cnidarians in the plankton is observed when the water column is homogeneous, or when stratification begins, at the end of spring (Primo et al., 2012).

Since 1994 until 2015, *M. atlantica* represented the most abundant gelatinous species (Azeiteiro et al., 1999; Marques et al., 2006; Primo et al., 2012), mainly during spring-summer periods. This siphonophore peak preceded the maximum abundance of hydromedusae, and it could be explained because of the formation of polymorphic colonies with high growth rates (Purcell, 1982).

In the last decades, the massive proliferation of gelatinous organism reported in many coastal areas has been linked with significant changes in the functioning of marine and estuarine ecosystems (Atrill et al., 2007; Condon et al., 2013; Lynam et al., 2004). In the north Atlantic there is a significant link between climate and gelatinous organisms´ abundance, however, no study had shown a clear correlation between gelatinous of the Mondego estuary and atmospheric weather patterns, such as North Atlantic Oscillation, and no mechanism had been proposed to link gelatinous abundance to climate change.

In the Mondego estuary, fluctuation in gelatinous blooms had been suggested to arise from temperature (Primo et al., 2012), mostly during last years, when this system has been affected by extreme climate events, such droughts that strongly occurred during the years 2004-2005 and 2007-2008. These events promoted the entrance of marine species in the estuary, with consequent alterations in the structure and dynamics of the zooplankton community (Marques et al., 2014; Primo et al., 2011).

In estuaries, physical conditions respond at different time and space scale to the external physical forces, such as freshwater flow, wind and ocean conditions (e.g.

tidal movement, mean sea level, salinity) (Kimmerer, 2004). In eastern ocean margins, such as in the Iberian Peninsula, vertical and horizontal distribution abundance of gelatinous carnivores are affected by North Atlantic climate variability (Pavez et al., 2010). Particularly, these forces can influence the upwelling process, primary and secondary productivity by the nutrient-rich upwelled waters (Alvarez et al., 2008; Miranda et al., 2013), affecting the distribution of siphonophores as happened, for example, in Campeche Bank (Mexico), particularly during the spring (Gasca and Suarez, 1991), the species vertical distribution in Monterey Bay, California (Silguero and Robinson, 2000), or the abundance of hydromedusae in the Tropical East Pacific (Miglietta et al., 2008).

In the Mondego estuary, after 2007-2008, the higher variation and the increase of the upwelling activity, mostly influenced by the North Atlantic Oscillation, have influenced species richness and phenology of gelatinous carnivores. These phenological changes, that may influence the structure and the diversity of zooplankton community, were clear in the results of the study over the period 2003-2013, observing a shift from unimodal to bimodal peak in the mean annual pattern of gelatinous carnivores, prior and after 2007 respectively.

5.2 *Muggiaea atlantica* and *Muggiaea kochii* in the Mondego estuary

In contrast to hydromedusae, the siphonophore life-cycle is entirely pelagic (Bouillon et al., 2006), increasing its density and occurrence in zooplankton. They are widespread in the oceans and the majority of the species are mainly referred as truly oceanic (Pugh, 1989), however, its presence has already been detected also in coastal, bays and estuarine waters (e.g. Molinero et al., 2005; Sanvicente-Añorve et al., 2009). This can be explained by their higher vulnerability to currents and water mass movements that may result in higher transport inside the estuary.

Whist other species fail to adapt to the change in environment; the siphonophores have less competition when foraging for food. Further, intense predation by *M. atlantica* can deplete prey resources, restricting the available energy for other functional groups and disrupting the balance of the ecosystem (Greve, 1994; Kršinić and Njire, 2001).

Much of our knowledge of siphonophores morphology and life cycle dates from the last years of the past century (Carré and Carré, 1991; Mackie et al., 1987; Pugh, 1989). However, little attention was given to the ecology of siphonophores, particularly in coastal ecosystems as estuaries. During the late 20th century, expansion of the *Muggiaea atlantica* distribution has been report in a number of different regions including the Mediterranean (Licandro et al., 2012; Batistić et al., 2013), Western English Channel (Blackett et al., 2014) and South Pacific (Palma et al., 2014).

In the Mondego estuary, *M. atlantica* represents the bulk of the gelatinous carnivorous community (D'Ambrosio et al., 2016; Marques et al., 2006; Primo et al., 2012, 2009), and its occurrence is favoured by the seawater penetration in the estuary. This species is frequently distributed at the most downstream regions of neritic ecosystems (Batistić et al., 2013; Blackett et al., 2015; Licandro et al., 2012), or in the Bight (Luo et al., 2014; Pavez et al., 2010), and its presence is related to increased primary and secondary production (Guerrero et al., 2016).

In recent decades, several studies about gelatinous species in the Mediterranean Sea (Guerrero et al., 2016; Licandro et al., 2012), showed how *M. atlantica* easily has expended its distribution range, become integral part of the zooplankton communities in warmer waters, which, until a few decades ago, were characterized by a greater abundance of the congener *M. kochii* (Batistić et al., 2007). Appropriate hydrographic conditions and the direction of surface currents, made possible its immigration into different neritic systems (Batistić et al., 2013; Greve, 1994), and establish new populations, as happened in the Western English Channel (Blackett et al., 2014) or in the Gulf of Mexico (Martell-Hernández et al., 2014).

In the Mondego estuary, *M. atlantica* population was regularly developing after 1994, and showed a longer bloom period (April-August) (Primo et al. 2012), with a progressive expansion of its seasonal abundance (Marques et al., 2006; Primo et al., 2012). This species reached the maximum historical abundance in the summer of 2009. In contrast, from the late 2012, the occurrence of *M. kochii* became constant, and its abundance grew during the last 5 years.

Regarding the distribution of the *Muggiaea* species, salinity and temperature have been pointed out as the main drivers responsible for this pattern (Blackett et al., 2014; Licandro et al., 2012; Lucas et al., 2014), and the latter one is considered a determinant key of reproduction in *Muggiaea* (Purcell, 1982; Carré and Carré, 1991).

In the Mondego estuary, temperature followed a seasonal pattern, presented highest values in the summer time (±25ºC max), and lowest values in the winter (ca 12ºC), but never below 11ºC. Thus, the density of the *Muggiaea* gonophores was greater during spring-summer, followed the same pattern of the *Muggiaea* polygastric stage. The higher temperature caused the fast release of the eudoxids from the colonies, and faster maturation of the gonophores (Purcell, 2005), meaning that in the Mondego estuary, these two species found suitable thermal conditions for reproduction. In the Mediterranean Sea, Batistić et al. (2013) and Licandro et al. (2012) associated the highest abundance values of this species at a temperature range between 13 and 16 degrees, while in WEC, Blackett et al. (2015) suggested a temperature of 10ºC as the limit for *Muggiaea*'s asexual reproduction. In addition, the results presented on this dissertation, explained that, on long, seasonal and daily scale, salinity was a remarkable driver that most affect the distribution, abundance and the entrance of *Muggiaea* species in the Mondego estuary.

Several studies have pointed out that the salinity is one of the variables strongly related to the distribution pattern of this species in different neritic systems, for example, in the North West Mediterranean (Gili et al., 1988; Guerrero et al., 2016; Licandro et al., 2012) or in the North Atlantic (Blackett et al., 2014).

In the Mondego estuary, the prevalence of marine conditions in the estuary, as the increase of salinity due lower precipitation regime, as happened during 2015, caused the rise in the estuary of the two species. In addition, in a dryer year, the number of colonies of *M. atlantica* have undergone a huge increase. In contrast, the abundance of *M. kochii* seemed not been affected by increased salinity, and the number of its colonies in 2015 (dry year) remained similar at 2014 (regular year).

Furthermore, it has been shown that the ecology of siphonophores depends, also, from food availability, being *Muggiaea* species considered voracious predators in surface waters, being able, during its blooms, to remove a substantial proportion of prey, causing changes in the density of zooplankton communities (Greve, 1994;

Mills, 1995; Purcell, 1982). Moreover, the number of eudoxids produced by *M. atlantica* increased rapidly with the amount of food and rapidly vanish in case of fasting (Dallot et al., 1988; Purcell, 1982).

The study on the gastrozooids content of the *Muggiaea atlantica* eudoxids, showed the selectivity of this species in terms of prey, confirming that copepods and their developmental stages, as copepods nauplii, represent the dominant dietary component of *M. atlantica* (Licandro et al., 2012; Mapstone, 2009; Purcell, 1982). In the study on the feeding behavior of *M. atlantica*, the most abundant prey found in the gastrozooids were the harpacticods *Euterpina acutifrons* and copepods nauplii, that represented the most abundant and the smallest prey in the Mondego estuary. Unlike the siphonophores with larger stomachs fed fish larvae, shrimps or ostracods (Purcell, 1983), *Muggiaea atlantica* and other calycophoran species, as *Diphyes dispar* (Purcell, 1983), due to the reduced size of their stomachs, fed only smaller organisms, such copepods.

5.3 Limitations of the study

I acknowledge that there are some caveats in the investigation discussed in this dissertation, especially in the studies presented in the second and third chapters. The primary limitation to work with gelatinous zooplankton in general, and with siphonophores in this particular case, and the reason that so little is known about them, is the extreme difficulty to collect their colonies intact. In most of cases, this restriction causes under estimations of abundance and distribution of siphonophores, both polygastric (asexual) and eudoxids (sexual) stages. In the case of this study, in the second chapters, some samples were found damaged and it was impossible to identify the nectophores between the two species. Moreover, for the same reason, in the third chapter we managed to analyse only the stomach content of the intact eudoxids, but unable to estimate predation rate and food selectivity of adult colonies.

Second, *M. atlantica* and *M. kochii*, as this thesis suggested, cohabit in the Mondego estuary, though the former more abundant than the latter. Being the eudoxids produced by the two species morphologically indistinguishable, it was not viable to demonstrate which of them produced more eudoxids.

Third, the study of the third chapter was conducted only for a half-tidal cycle in day time. Thus, the results might have been affected, especially considering prior studies that have described greater abundances of *M. atlantica* and higher predation rates during night time.

Fourth, to identify the prey found in the gastrozooids of the *M. atlantica* eudoxids, we were only based on visual identification. Therefore, we were able to estimate only predation rates of the undamaged prey, unable to include unidentified stomach content in the analyses.

5.4 Future research

This thesis synthesizes valuable information about the gelatinous zooplankton community in the Mondego estuary, documented how climatic variability affects the abundance, distribution and the phenology of this organisms, creating a cascade effect that influence the structure and dynamics of the Mondego ecosystems. Moreover, the present study has clearly detected the crucial ecological role of the *M. atlantica* and *M. kochii* in this ecosystem, and how its blooms may influence the structure of zooplankton community, particularly the abundance of their prey.

Thus, to estimate more appropriately the predation impact of the siphonophores in estuarine and coastal systems, future studies are needed to gather better knowledge about the feeding habits and the predatory mechanisms of siphonophores, as well as the dynamics of their main prey organisms. Moreover, further research wanting to describe trophic links and investigate dietary behaviour of the species through the observation of the stomach content should be supplemented by biochemical markers that provide accurate information on trophic links (e.g. fatty acids, isotopes analysis, molecular markers).

Particularly, the increase of salinity, during a low precipitation regime, is the main driver that influenced the increase of the abundance of *M. atlantica*. Thus, if the drought events scenario persists, the abundance of this important predator may grow in coastal and neritic ecosystems, with direct influences on the zooplankton communities and indirect effects on higher trophic levels. Further *in situ* observations are required in order to gain a better understanding of the relationship between the abundance of siphonophores in neritic areas and physical environmental factors. More long-term ecological studies will also be important to a better understanding of its immigration in different coastal and estuarine ecosystems.

The biology of these two species is still poorly known. These species are easily handled in the laboratory, and Carré and Carré (1991) have been able to describe the life cycle M. kochii at different temperatures, but a similar work is not still available for *M. atlantica*. Moreover, an additional impediment is the fragility of these organisms and the unappropriated methods use to collect intact colonies.

"Some forms of sea life are simply too fragile to be extracted from their supportive, watery environment" (Bruce H. Robison, 1995).

For a better knowledge on the morphology of these colonies, apart from the direct scuba diving observation, it would be important to develop a less invasive sampling method to collect untouched colonies.

Gelatinous zooplankton plays major controlling roles in marine ecosystems (as trophic dead-ends) and, in this era with a scenario of apparent jellyfish dominance, marine ecosystem managers and modelers cannot afford to ignore them.

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AGRADECIMENTOS

Parece que está!

Um profundo obrigada:

- Aos meus orientadores, os Professores Miguel Pardal e Ulisses Azeiteiro, para me orientar e ajudar neste caminho.
- Á minha orientadora, mas mais do que isto, amiga, Sónia, para me aguentar e ajudar imenso. Agradeço-te muito porque foste tu que me insinaste tudo aquilo que sei, e que me abriste as portas do mundo planctónico. E adorei, fiquei logo apaixonada por estes bichinhos! Muito obrigada, do profundo do coração.
- Thank you so much to Juan Carlos Molinero, for taking care so good of me in Kiel, for all the lunch-dinner times there, for our "ecological" conversations on Skype util 3am.
- Á Joana Cruz e á Elena Guerrero, agradeço o tempo gasto comigo em Barcelona ou em Faro. Obrigada pelos conselhos e a amizade.
- Obrigada Lígia, chatiei sempre muito, eu sei, ainda me lembro do dia em que ganhei a bolsa de Doutoramento. Liguei logo para ti, não acreditava! Obrigada pelo apoio e pelos imensos conselhos.
- Á Filipe e Daniel, sempre soube que podia contar convosco, por qualquer coisa, seja que for. Obrigada Filipe para conducir tão bem o Darwin I 😊.
- Um profundo obrigada também a Patrícia. Foste tu que me disseste um dia: "anda lá, quem corre uma maratona consegue tudo! Escrever uma tese é mais simples". Mais ou menos 😊..
- Agradeço também o resto do grupo com quem partilhei inúmeros momentos nestes quatros anos: João Rosa, João Rito, Joana Baptista, Joana Oliveira, Sara, Elsa, Joana Falcão, Ivan.
- Agradeço a senhora Lina da biblioteca, pelos sorrisos e os docinhos que sempre ajudaram.
- Aos amigos do Fit and Fun, sobretudo ao João Afonso e Joana Mendes. Um obrigada também aos companheiros de imensas corridas que me proporcionaram tanto bom estar e muitas endorfinas 😊.
- Um beijinho vai á Claudia e ao Nuno, á Cristina, minha Duartinha, á Susana, á Ângela e Ugo. Obrigada amigos, vocês são o maxímo.
- Agradeço do profundo do meu coração á minha família portuguesa: Anabela, Fausto e António. Obrigada para tratar de mim tão bem, para eu me sentir como uma filha, para todos os almoços e jantares a quem me convidaram (e a quem me convidei), para os momentos felizes. Um grande pedaço deste trabalho é para vocês.

- Ao meu amigo do coração Tiago. Ahi quantas telefonemas a qualquer hora!!! E quantos momentos passados juntos! Obrigada por tudo, tu sabes, não preciso escrever. Gosto de ti.
- Á minha Evuccia…sem ti isto não era possível. Ou pelo menos, era, mas sem tantas gargalhadas. Toma lá um ♡ 😉. Obrigada Lolita, irmãzinha pequena.
- Então Nevesinho, agora era suposto que conseguisse agradecer-te com duas linhas? Eu nem sei para aonde começar! Tu também sabes, vocês os três sabem perfeitamente que não há palavras que consigam explicar o que passamos juntos. Muito obrigada, para me aturar tão bem, eu não sei se me aguentava. Vocês são especiais. 😊 Muitos momentos partilhados, muita vida juntos..tão bom isto!
- Á minha companheira de muitas noites de escrita louca, a minha pequena Sushi, a minha sombra lá em casa, sempre ao meu lado. A dormir a maioria das vezes, mas sempre ao meu lado 😊.
- Minha Mariuxi Pocahontas, consideras os agradecimentos um capitulo ou um subcapítulo? Eu os considero um paragrafo, claro! 😉 Esta sim que foi uma viagem de loucura! Vamos sempre lembrar-nos deste momentos, mas foi ótimo partilha-los contigo. Ganhei uma amiga do coração, e só por isso valeu a pena meter-se nesta maluquice. Como tu sempre irás te lembrar da tua italiana doida! Agradeço a tua presença, foste muito importante nesta reta final.
- Obrigada Max, quanta chocolate comemos na sala? Se encontrar um colchão velho, eu aviso, tranquilo!
- Obrigada António, pela maravilhosa capa e por tudo. Sobretudo pelos sorrisos, tão preciosos nesta fase de doidice. "L'universo non ci castiga".. 😉

Passando all'italiano:

- Grazie alla mia famiglia, per essermi stata cosí d'aiuto, ma questo sempre, non solo in questo processo. Spero di rendervi orgogliosa. Ringrazio i miei nipoti Alessio e Maristel per i sorrisi dolci che mi hanno riempito il cuore in momenti un pó cosí.
- Un grazie speciale va ad Elisa e Carla..ma ancora mi volete bene? Dopo tutti i miei: Andiamo, andiamo, Facciamo, Corriamo! Io vi voglio bene, si…anche perché non posso darmi il lusso di non farlo, sono cosí poche le persone che mi sopportano. Elisa, questa volta senza Lucano, che tristezza immane!
- Un bacio grande alla mia Adri e al mio Gegio 😊

Espero de não ter esquecido de ninguém, a primeira copiá mais fofinha e cuidadosa dos agradecimentos foi perdida no meio das centenas de copy-paste desta escrita. Peço que desculpem os erros de português, depois de tanto tempo ainda não aprendi a escrever um correto português, preciso de mais anos aqui 😊.

Um profundo obrigada a todos!

Mariaelena