Spatial distribution and seasonal patterns of the siphonophores *Muggiaea atlantica* and *Muggiaea kochii* in a temperate estuarine ecosystem

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A B S T R A C T

This study analysed the distribution and abundance of both polygastric (asexual) and gonophores of the caly-cophoran siphonophores *Muggiaea atlantica* and *Muggiaea kochii* in the Mondego estuary (Portugal), as well as the influence of local environment and prey availability on the observed patterns. Samples were collected in three different zones of the estuary, from December 2013 to November 2015. In general, both species nectophores and total gonophores followed a similar trend, with higher abundances during spring-summer periods and increased densities in the downstream areas of the estuary, where the marine influence is stronger. Inter-annual differences occurred mainly for *M. atlantica* nectophores, which showed higher abundances in 2015, particularly in the upstream areas. Both species displayed a shift in the annual peak abundance, from bimodal in 2014 to unimodal in 2015. Patterns observed were not only strongly related with an increase of the marine influence in the estuary, but also with the presence of zooplanktonic prey, such as cirripede *cypris*, calanoid *nauplius*, Poecilostomatoida, and copepodites of Cyclopoida. This study represents a step towards a better knowledge of the biology and ecology of siphonophores.

1. Introduction

The hydrozoan class of Siphonophora represents one of the major groups of the zooplankton (Carré and Carré, 1991; Pugh, 1989), which is constituted by pelagic, benthonic, and neritic free-swimming colonial species (Mapstone, 2014; Pugh, 1974). Siphonophores have a relevant ecological role since under appropriate environmental factors, such as temperature, salinity and food availability, these organisms are able to bloom (Blackett et al., 2015; Lucas et al., 2014). As voracious plankti-vorous predators, they can significantly affect prey populations such as 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). Eudoxids are free-swimming cormidium able to bud into several gonophores that, via external fertilization, generate a new polygastric colony (Carré and Carré, 1991; Mapstone, 2014). *Muggiaea kochii* is a very common Mediterranean species with a preference for relatively high-tempera-ture waters (> 13 °C) (Batistić et al., 2013); however, its presence has also been detected 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 gela-tinous 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 one of the major components of the neritic gelatinous zooplankton (Mapstone, 2014). It is a cool-temperate species, typical from Atlantic waters (Bouillon et al., 2004 and references therein) that is able to complete its life cycle at lower water temperatures (Batistić et al., 2013). Since the 80's, *M. atlantica* has established new populations in the Western English Channel and progressively colonized the Western Mediterranean, which lead to the displacement of the native population of *M. kochii* in the Adriatic Sea (Batistić et al., 2007; Blackett et al., 2014).

In the Mondego estuary, a temperate shallow system in the western

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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) showed a decrease of this species in the estuary from 2004 to 2009, when its abundance bloomed again. On the other hand, *M. kochii* was considered a rare species in the estuary until 2012, when its abundance increased, and has been a common gelatinous species in the estuary since then (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 these gelatinous carnivore zooplankton (D'Ambrosio et al., 2016; Lynam et al., 2005). However, siphonophore annual patterns in temperate shallow systems, such as the Mondego estuary, are still poorly known. In addition, there is a lack of studies focusing on the range of ecological conditions (e.g. temperature and salinity) suitable for their sexual reproduction in natural environments, which is essential for predicting the establishment and invasion of new areas, as well as changes in their abundance under global climate change scenarios. For instance, recent studies in the Mondego estuary showed how the prevalence of marine conditions promoted the entrance of marine species, including gelatinous carnivore zooplankton (e.g. Baptista et al., 2015; Marques et al., 2014; Primo et al., 2015, 2011). This highlights the suitability of this transition area as a natural laboratory, providing the opportunity to study the influence of environmental variability in the life cycle and ecological interactions of these species.

The present study aims to characterize the annual abundance patterns of the congeneric species *M. atlantica* and *M. kochii* in the Mondego estuarine ecosystem and assess the influence of environmental conditions in their ontogenic development. For that, changes in abundance and spatial distribution of polygastric stage and gonophores of both species during two consecutive years were analysed and their relationship with local environmental conditions investigated.

2. Materials and methods

2.1. 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 and join again near the mouth (Fig. 1). The two arms are separated by the Murraceira Island and are characterized by different hydrological characteristics, being the northern one deeper (4–8 m deep at high tide; residence time < 1 day) than the southern one (2–4 m deep at high tide; residence time < 3 days). A complete description of the system can be found in Marques et al. (2006).

2.2. Sample collection

Muggiaea species were collected once a month between December 2013 and November 2015 at high tide in six sampling stations in the Mondego estuary (M1 e M2 – mouth; N1 e N2 – northern arm; S1 e S2 – southern arm) (Fig. 1), as a part of a long-term zooplankton research program that began in 2003 (Marques et al., 2014). The sampling period was divided into 2 years from December 2013 until November 2014 - first year of the study, and from December 2014 until November 2015 – the second one. To simplify, we refer to the first year as 2014 and to the second year as 2015.

Samples were collected by subsurface horizontal tows (10 cm below surface), using a plankton net (open diameter 0.5 m, mesh size 335 μm, tow speed: 2 knots, tow length: 3 min, 1 replicate per station) and immediately fixed with 4% borax buffered formalin. The volume of filtered water was estimated using a Hydro-Bios flow meter mounted in the opening of the net (average 39 ± 22 m³). In the laboratory, siphonophores were separated, identified and counted under a dissecting microscope. Abundance was estimated and expressed as individuals per m³. Since *Muggiaea* species only develop a single nectophore (Mackie et al., 1987) the anterior nectophores of both *M. atlantica* and *M. kochii* were used to estimate the abundance of their asexual polygastric stage (Fig. 2A and B). In addition, they also develop free-living eudoxids (sexual stage), which produce a succession of gonophores that ripen and release gametes (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 between the two (Carré and Carré, 1991). Since gonophores of the genus *Muggiaea* are morphologically indistinguishable (Kirkpatrick and Pugh, 1984), female and male gonophores of both species of were identified and counted together (Fig. 2C and D).

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

Simultaneously to each zooplankton sampling, the surface temperature and salinity were recorded *in situ* using a WTW Cond 330i portable meter. As no sampling was made in January 2015, the temperature and salinity values recorded during January 2014 were not considered for the mean calculations. 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 Portuguese Sea and Atmosphere Institute (www.ipma.pt).

2.3. Statistical analysis

Differences in salinity among sampling stations were tested with one-way ANOVA, and then grouped into zones accordingly. Hence, three different zones were constituted: Z1, mouth and middle south arm (M1, M2, S1), characterized by higher marine influence and salinity; Z2, middle north arm (N1), the transition zone influenced by the seawater and the freshwater discharges from Mondego River; and Z3, the upper estuary (N2 and S2), characterized by the lowest salinity values (Fig. 1).

The relationship between environmental conditions and *Muggiaea* spp. abundances was established with generalized linear models (GLM, family: Gaussian, link: identity). Models tested whether the independent variables water temperature, salinity, precipitation, zone and concentration of prey items were significant predictors for the log(x +1) transformed densities of *M. atlantica* and *M. kochii* nectophores and *Muggiaea* spp. gonophores, separately. Abundances of prey items were also log(x+1) transformed. Tests were performed for each year, individually. The collinearity among predictor variables was evaluated by pair-wise (Pearson) correlation coefficients (r) and Variance Inflation Factors analysis. Variables with r-values > 0.8 and/or VIF > 3 were removed from the analysis: zone was highly correlated with salinity, as well as copepodite stages (CI-CV) of Calanoida with Calanoida adults and Harpaticoida. Thus, zone and copepodite stages (CI-CV) of Calanoida were removed and their redundant influence was considered. The selection procedure used was the forward selection routine based on Akaike's Information Criteria (AIC). Then drop1 routine (Chambers, 1992) was applied to test the significance of each variable and reduce the number of variables. Models with similar number of variables and AIC were compared by ANOVA and the one with lower number of
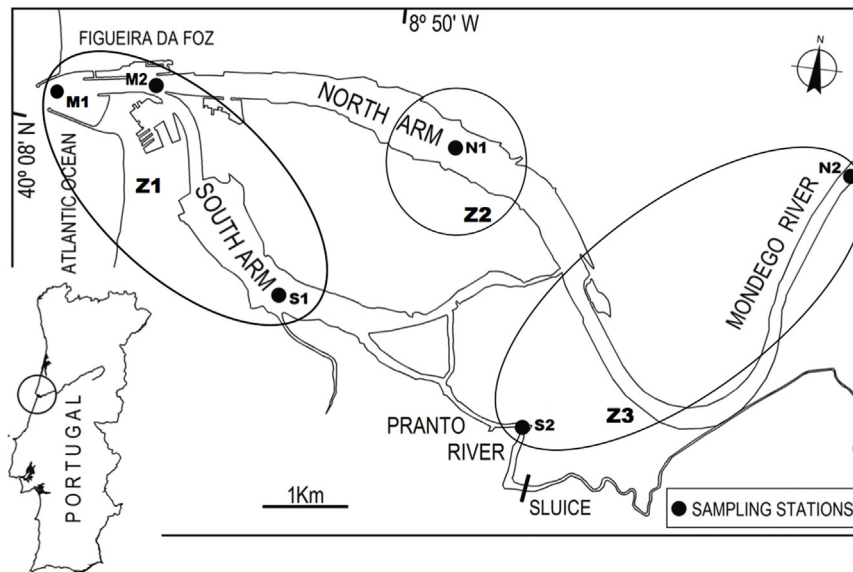
Fig. 1. Geographical location of Mondego estuary and the six sampling stations (M1, M2, N1, N2, S1, S2) grouped by the three zones (Z1, Z2 and Z3). Please see text for more details.
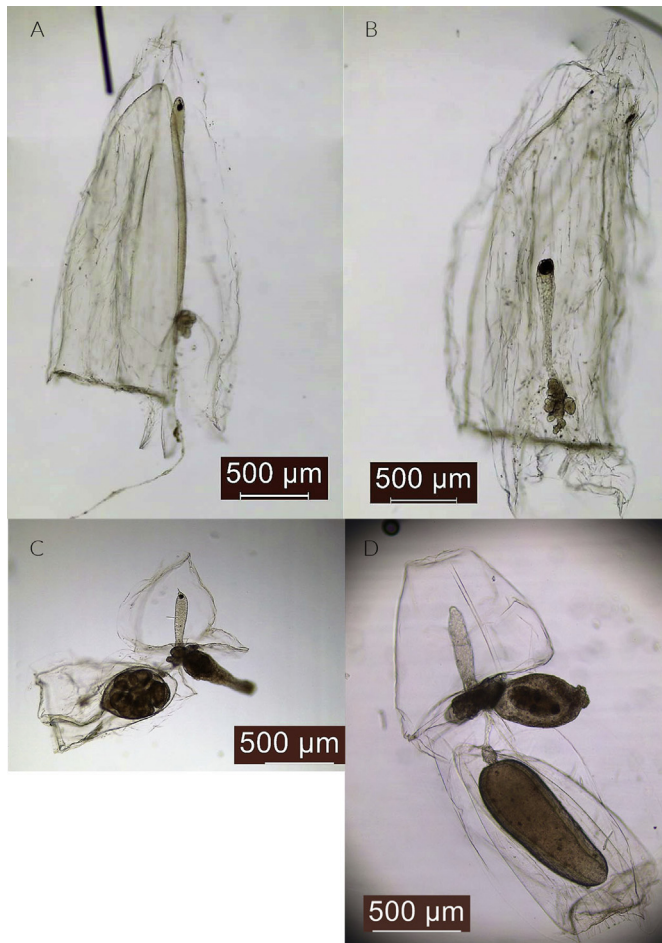
Fig. 2. *Muggiaea atlantica* anterior nectophore (A); *Muggiaea kochii* anterior nectophore (B); *Muggiaea* spp. eudoxid with a female gonophore (C); *Muggiaea* spp. eudoxid with a male gonophore (D). Credits by João Neves.

significant predictor variables, yet not statistically different from the other, was considered the best fitted model. The statistical analyses were performed using R 2.10.0 (R Core Team, 2014).

3. Results

3.1. Environmental parameters

During the two-year period, water temperature followed a similar seasonal pattern with higher values during summer (25.6 °C, Z3 on July 2015) and lower in the winter (11.1 °C, Z2 on February 2014) (Table 1; Fig. 3A). Z1 and Z2 presented similar values, whereas Z3 showed higher values during summer and slightly lower in the winter.

Precipitation in the Mondego estuary area also showed a typical seasonal variability, with higher rainfall during autumn and winter months and lower during summer (Fig. 3B). Compared to the mean precipitation regime for central Portugal during the 1981–2010 period, 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 rainfall peaks were observed in the spring (April, 54.3 mm; May, 51.9 mm) and autumn (October, 94 mm), and the lowest in summer (July, 0.5 mm).

The annual trend for salinity in the estuary presented clear differences between the two studied years (Fig. 3C; Table 1). In 2014, two abrupt low salinity events, reaching zero values in the three zones, occurred in winter (January–March) and autumn (November), while the maximum was during summer (August, 39.05). In 2015, zero salinity values were never observed, and a more stable water column was found in the estuary: the lowest values occurred during the winter (February, 7.4) and the highest in the summer and autumn (May and July, 34.1; September and November, 33.9, respectively) (Table 1). Areas Z1 and Z2 showed similar salinity along the studied period, being Z2 more variable than Z1; Z3 always presented lower values than the other two zones (Fig. 3C; Table 1).

3.2. Temporal and spatial variation of M. atlantica and M. kochii nectophores and Muggiaea spp. gonophores

Nectophores of both *Muggiaea* species followed similar seasonal trends, with the highest abundance during the summer period and the lowest during the winter. Nectophores showed seasonal inter-annual differences: in 2014, it was observed a bimodal trend in abundance with maximum peaks in early summer (June) and in autumn (October),

Table 1

Mean ± SD and minimum-maximum range of temperature and salinity registered in the Mondego estuary during the studied period in the three sampling zones. Temperature and salinity from January 2014 were excluded from the calculation of the means.

	2014			2015		
	Z1	Z2	Z3	Z1	Z2	Z3
Temperature (ºC)						
Mean ± SD	16.7 ± 2.7	17.0 ± 3.1	19.3 ± 4.8	16.8 ± 2.4	16.7 ± 2.6	19.0 ± 4.8
Min-Max	11.4–20.8	11.1–21	11.5–25	13.2–21	13–21	11.9–26
Salinity						
Mean ± SD	24.1 ± 11.6	20.2 ± 13.8	10.8 ± 7.5	29.9 ± 3.8	29.7 ± 3.9	16.8 ± 6.6
Min-Max	0.7–39	0–38	0–21	25–34	22.7–34	7.4–28

while in 2015 the general trend was unimodal for both species, with a summer peak (July) (Fig. 4). In 2014, both species presented similar monthly abundances with the exception of summer and late autumn months, when *M. kochii* was more abundant, which resulted in a higher mean annual abundance for this species (Fig. 4, Table 2). However, *M. atlantica* clearly dominated over its congeneric species throughout 2015, showing high annual mean densities when compared to 2014 (Fig. 4, Table 2). *Muggiaea* nectophores were distributed in Z1 and Z2 with similar abundance, although slightly higher in Z1. In contrast, Z3 always showed the lowest abundances (Fig. 4, Table 2). The three zones showed similar annual trends, with the highest abundance peaks in the same months, although with longer absence periods towards the upper estuary area (Z3) (Fig. 4).

Both male and female gonophores of *Muggiaea* spp. presented similar seasonal trends, mimicking those of the nectophores, with maximum values in summer and minimum in winter (Fig. 4). They also presented a bimodal distribution in 2014 and a unimodal one in 2015. Both male and female gonophores presented similar abundances in 2014, but in 2015 female gonophores were more abundant. *Muggiaea* spp. gonophores displayed a decreasing trend in abundance from Z1 to Z3, where they were virtually absent in 2014. Zones Z1 and Z2 showed similar 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 were present in the summer and early-autumn of 2015 (Fig. 4, Table 2).

3.3. Temporal and spatial availability of the potential prey

Calanoids and cyclopoids were the most abundant groups in the zooplankton (Fig. 5), representing between 33% (winter of 2014 in Z1) and 92% (spring of 2015 in Z3) of the total prey percentage for the first, and between 3% (summer of 2014 in Z3) and 54% (winter of 2014 in Z2) for the latter. Summer was the season with higher prey densities, mainly calanoids and cyclopoids, with the highest abundance peaks in 2014 for Z1 (ca. 10000 ind/m³) and in 2015 for Z2 and Z3 (ca. 16000 ind/m³ and 17000 ind/m³, respectively), while lower abundances and a higher predominance of cyclopoids were observed in the winter.

Comparing with copepods, cirripedes showed lower abundance (max 300 ind/m³ for Z1 and Z2 and a peak of 1100 ind/m³ during the summer of 2015 for Z3) (Fig. 5). The two groups displayed a similar abundance pattern, though cirripedes presented another peak in Z2 during the winter of 2015 (Fig. 5). The most upstream area (Z3) presented the highest prey abundance, which was largely constituted by calanoids, with relative values between 49% (winter of 2014) and 93% (summer of 2015). Harpacticoids showed maximum abundances in the winter of 2015 in Z1 (34%), while poecilostomatoids had the highest relative abundances in the autumn of 2015 in Z1 (7%) and in the summer of 2015 in Z2 (5%) (Fig. 5).

Comparing both years, 2015 was characterized by an increase in abundance of the marine groups, e.g. harpacticoids, poecilostomatoids and cirripedes, and this was observed mostly during winter months (Fig. 5). Moreover, in Z1 the total abundance of prey was higher in 2014 (ca. 10000 ind/m³), whereas in Z2 and Z3 increased in 2015. (ca. 16000 ind/m³ and 17000 ind/m³, respectively) (Fig. 5).

3.4. Relationships between biological and environmental factors

In 2014, the selected model included salinity and cirripedes *cypris* as important factors driving the abundance trends of the nectophores of both *M. atlantica* and *M. kochii* (52% variation explained). For the gonophores, the model included cirripede *cypris* and nauplius of calanoids (61% variation explained). The positive regression coefficients for all explanatory variables indicate that the abundance increases with increasing salinity and abundance of calanoid *nauplius* and/or cirripede *cypris* (Table 3).

In 2015, the best-fitted model selected included salinity and Cyclopoida copepodites as significant factors affecting *M. atlantica* nectophores abundance, which explained 48% of the observed variation (Table 3). On the other hand, the model selected for *M. kochii*, included only Poecilostomatoida as a significant factor affecting the abundance of nectophores, explaining 28% of the variation observed for this species (Table 3). For gonophores, the best-fitted model included salinity and Cyclopoida copepodites, which explained 24% of the variation observed (Table 3). As in 2014, the influence of the factors included in the models in 2015 was positive, denoting a concurrent increase of the selected factors and *M. atlantica* and *M. kochii* nectophores and total gonophores (Table 3).

As the results of the regression suggest, salinity was the most significant factor explaining the abundance and distribution of nectophores and gonophores during the study period, except for *M. kochii* nectophores in 2015 and for the gonophores in 2014, which were mainly driven by their food sources. Despite that the factor Zone was removed from the models due to collinearity with salinity, a significant influence of the latter implies a significant effect of the area of distribution within the estuary, indicating that the distribution of *M. atlantica* and *M. kochii* nectophores and female and male gonophores showed an important spatial differentiation.

4. Discussion

Muggiaea atlantica and *M. kochii* nectophores and gonophores presented similar seasonal and spatial patterns in the Mondego estuary, with the highest abundances observed during the warmer months. This was previously reported for the same species in the Western English Channel (Blackett et al., 2015, 2014) and in the Northwest Mediterranean (Gili et al., 1987; Guerrero et al., 2016). The increase of carnivorous gelatinous zooplankton is often related with an increase in sea surface temperature and zooplankton availability which trigger species reproduction, and result in higher number of organisms (Purcell, 2012; Robinson and Graham, 2013). Despite their eurythermal nature, both species seem to present a clear threshold for reproduction. For instance, Blackett et al. (2015) suggested a critical basal temperature limit of 10 °C for *M. atlantica* asexual reproductive activity in the English Channel, while Carré and Carré (1991) did not observe sexual
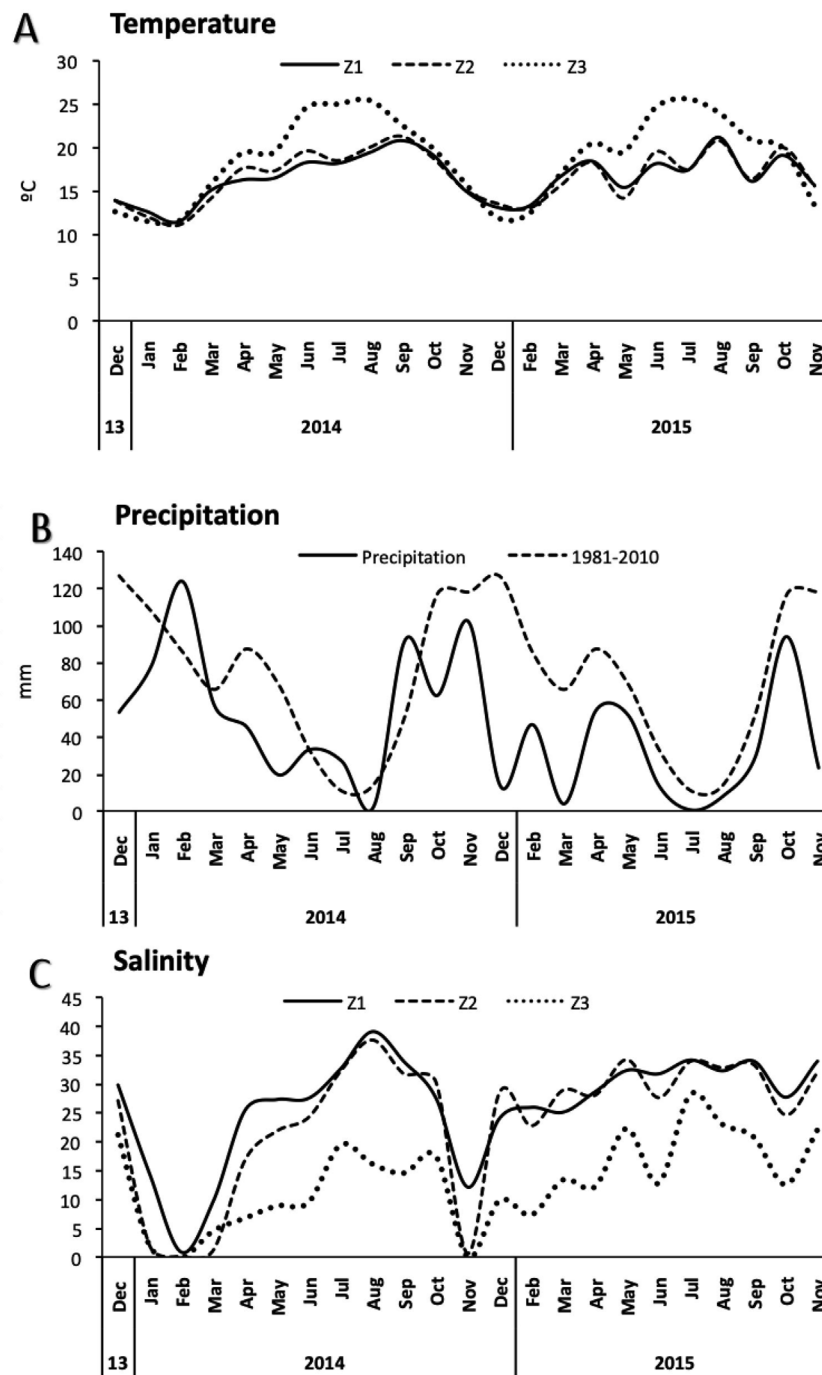
Fig. 3. Temporal variation of the environmental parameters in the Mondego estuary from December 2013 to November 2015. (A) Surface temperature in the three zones (ºC). (B) Precipitation values (mm) for the area (black line) and mean precipitation for the 1981–2010 period (dotted line). (C) Surface salinity in the three zones.

reproduction for *M. kochii* at temperatures below 13 °C. In this study, the presence *M. atlantica* nectophores and *Muggiaea* spp. gonophores pointed out 13 °C as the most probable lower limit observed for the sexual reproduction of this species in the estuary.

The distribution of both species in the estuary had a strong spatial component. The highest densities were recorded in the downstream area (Z1), mainly influenced by seawater, and the lowest ones in the upper estuarine area (Z3), which are markedly dominated by freshwater input. Previous studies have already demonstrated that in shallow estuarine systems such as the Mondego, gelatinous organisms are more abundant in the lower estuary, due to the marine influence (Marques et al., 2008; Pereira et al., 2014). *M. atlantica* and *M. kochii*

commonly present the greatest density at the mouth of rivers, due to the their euryhaline and eurythermal characteristics (Gili et al., 1987).

Inter-annual changes were mainly visible in the spatial distribution of *M. atlantica* nectophores, which showed higher abundances in 2015, particularly in the upstream areas. This increasing abundance was mostly related with the higher intrusion of the marine waters in the estuary, as a consequence of reduced rainfall, mainly in the winter. In fact, the observed increase in salinity in 2015 was similar to what had been previously recorded in the area due to the occurrence of extreme droughts (lower precipitation and freshwater flow), which led to a marinization of the ecosystem. This promoted the establishment of more marine species in the estuary and consequently affected the
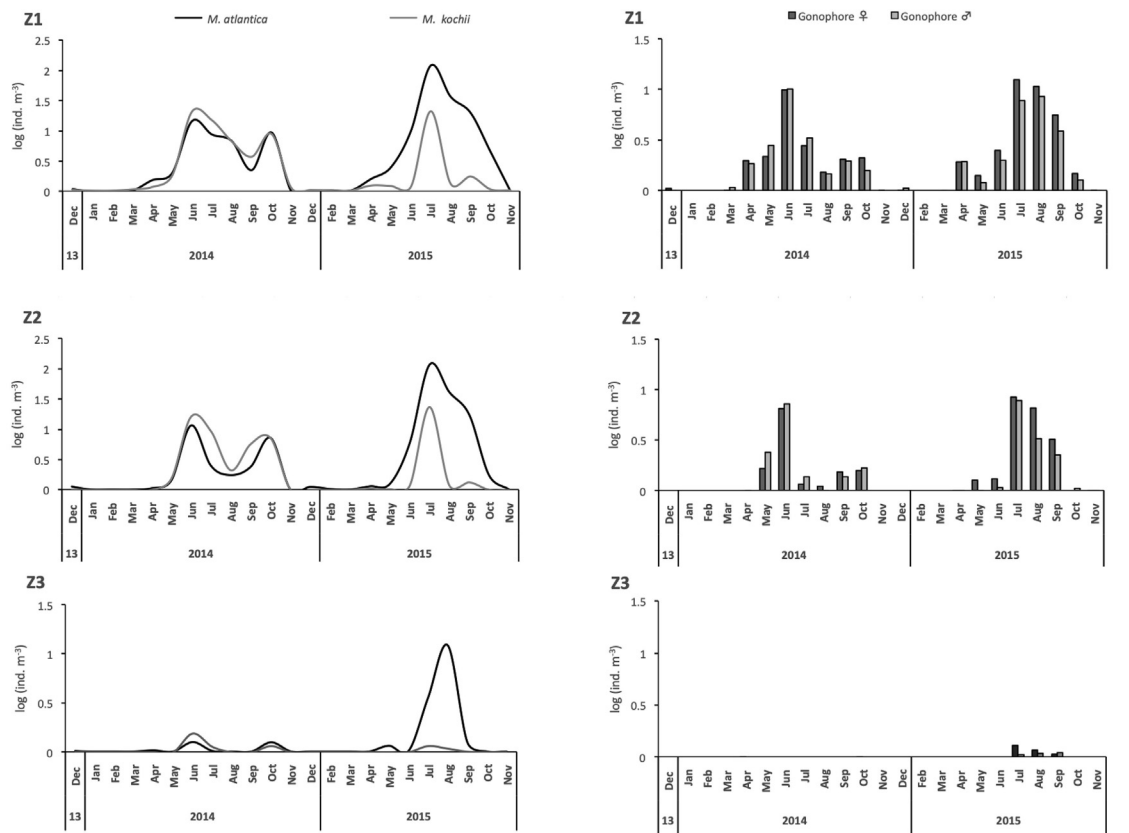
Fig. 4. Temporal fluctuations in the abundance (log-transformed data) of *M. atlantica* and *M. kochii* nectophores (lines, on the left) and *Muggiaea* spp. female and male gonophores (columns, on the right) (ind. m^{-3}) in the three zones.

Table 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
M. atlantica nectophores						
Mean ± SD	2.9 ± 4.2	1.6 ± 3.0	0.04 ± 0.09	18.8 ± 35.0	17.7 ± 34.6	1.3 ± 3.2
Max	13	10	0	119	115	11
M. kochii nectophores						
Mean ± SD	3.9 ± 6.0	2.7 ± 4.4	0.07 ± 0.1	2.1 ± 5.9	2.3 ± 6.6	0.02 ± 0.04
Max	20	15	1	20	22	0
Muggiaea spp. ♀ gonophores						
Mean ± SD	1.2 ± 2.3	0.6 ± 1.4	0	2.9 ± 4.1	1.6 ± 2.6	0.04 ± 0.09
Max	9	6	0	12	7	0
Muggiaea spp. ♂ gonophores						
Mean ± SD	1.2 ± 2.4	0.7 ± 1.6	0	1.9 ± 2.7	1.1 ± 2.0	0.02 ± 0.04
Max	9	6	0	7	7	0

structure and dynamics of the estuarine planktonic and fish communities (Baptista et al., 2015; Marques et al., 2014; Martinho et al., 2007; Primo et al., 2011). Concomitantly, this marine intrusion most probably fostered the abundance of *M. atlantica* in the estuary and allowed the species to reach the upstream areas. Siphonophores have been observed in most coastal areas, bays and estuarine waters (e.g. Molinero et al., 2005; Sanvicente-Añorve et al., 2009) and their high susceptibility to currents and water mass movements (Lopez-Lopez et al., 2013; Palma and Silva, 2006) may result in higher transport into the estuary during low freshwater inflow periods, as observed in 2015. 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). The increased abundance of these gelatinous predators can affect the entire pelagic communities since they have a crucial role in shaping estuarine and coastal

zooplankton communities (Pereira et al., 2014). Indeed, when they occur in large numbers, prey populations can be significantly affected (Purcell, 1982; Sabatés et al., 2010). For instance, an invasion of *M. atlantica* in the Adriatic Sea in 1997 impacted significantly on the structure, distribution and density of *nauplii*, copepodites and adults of small copepods (Kršinić and Njire, 2001), and whose cascading effects can be transferred into the upper levels of the marine food web. These blooms can also affect the population size of fish larvae, and consequently, fish recruitment (Hansson et al., 2005). The increase in abundance of *Muggiaea* spp. in 2015 can also be indicative of population growth within the estuary during the warmer months, when conditions are more suitable for reproduction of these species. In accordance, gonophores were periodically absent during the winter, but increased in late spring and summer months. This occurred mainly due to the controlling effect of water temperature on their developmental
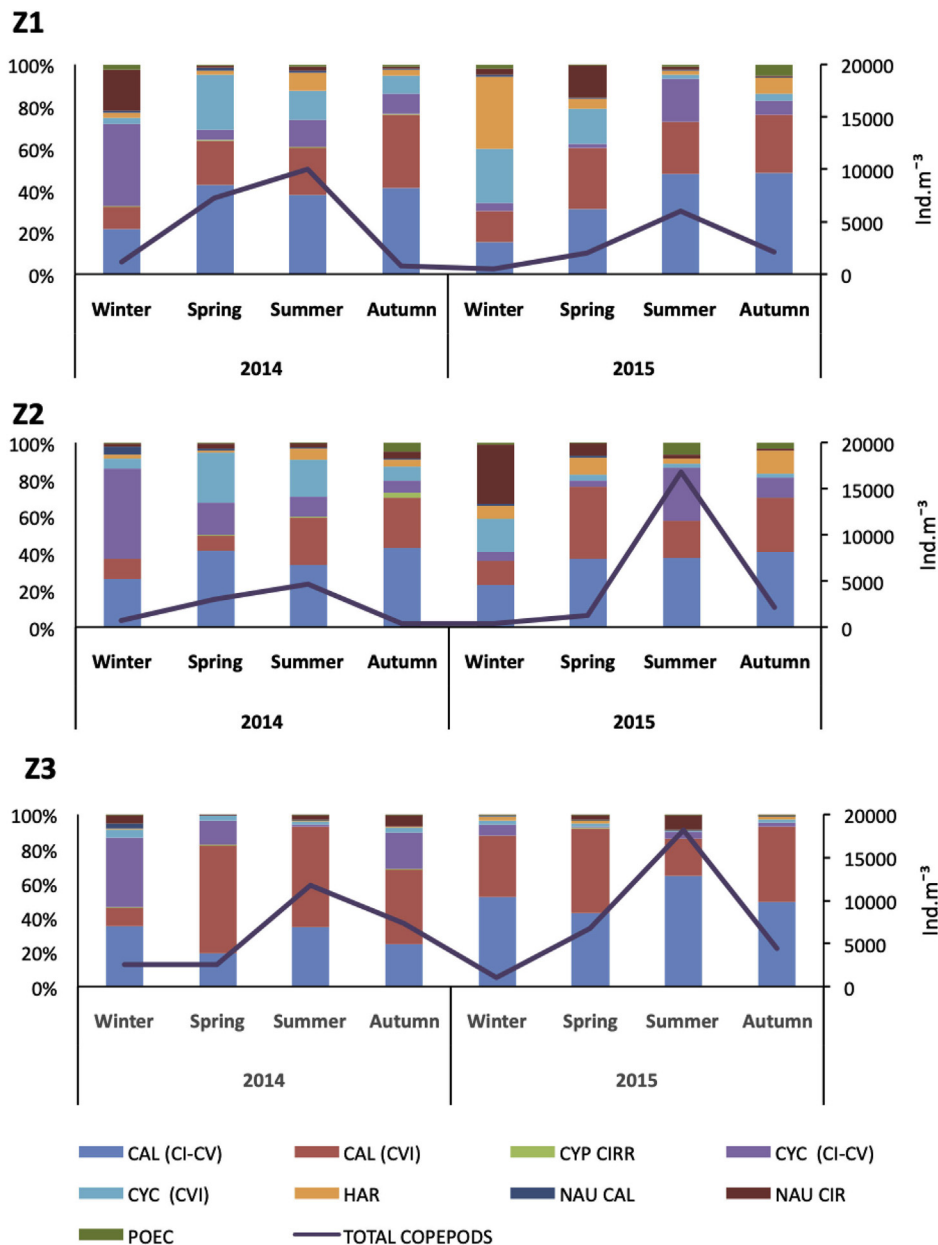
Fig. 5. Total (ind.m^{-3}, line) and relative abundance (%, bars) of the potential prey for *M. atlantica* and *M. kochii* at the three zones. CAL (CI-CV): Calanoida (CI-CV); CAL (CVI): Calanoida (CVI); CYP CIRR: Cypris Cirripede; CYC (CI-CV): Cyclopoida (CI-CV); CYC (CVI): Cyclopoida (CVI); HAR: Harpactacoida; NAU CAL: Nauplii Calanus; NAU CIR: Nauplii Cirripede; POEC: Poecilostomatoida.

rates: lower temperatures drive a slow maturation, while higher temperatures increase the release of eudoxids and a rapidly maturation of gonophores (Carré and Carré, 1991). The change in inter-annual seasonal distribution from bimodal in 2014 to unimodal in 2015 can be related with changes in water temperature, which is the main driver of gelatinous carnivore zooplankton blooms, as referred in former studies (Mills, 2001; Purcell, 2012; Robinson and Graham, 2013).

While both *Muggiaea* species cohabit in the estuary, their gonophores are morphologically indistinguishable (Kirkpatrick and Pugh, 1984), which made it impossible to determinate which species produced more eudoxids and gonophores. However, we accept that the larger proportion of the gonophores found in 2015 belonged to *M. atlantica,* the dominant species. As this species is able to sexually reproduce at lower temperatures, it has an advantage over its congener *M. kochii,* which is usually dominant in warmer temperate and subtropical waters of the Mediterranean and Atlantic Ocean (e.g. Martell-

Hernández et al., 2014; Nagata et al., 2014; Zakaria, 2004).

Our results pointed out salinity and prey abundance as the main environmental factors that affected the observed patterns of the two species in the estuary. Recently, salinity has been pointed out as the main driver responsible for the increase in *Muggiaea* spp. and other cnidarians in temperate areas (Blackett et al., 2014; Licandro et al., 2012; Lucas et al., 2014). Particularly in estuaries, salinity affects population size and asexual reproduction of cnidarians (Purcell, 2005), and can be considered as a proxy for physical processes, such as water circulation (Blackett et al., 2014). The strong influence of salinity in the present study is closely related with the previously mentioned intrusion of marine water and associated organisms in the estuary, which took place during lower freshwater inflow periods as summer and/or years with low precipitation regimes. This was evident in 2015, when the stable conditions allowed a more pronounced effect as mean salinity remained around 30 throughout the year in the more downstream areas

Table 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 Cirripede; CYC (CI-CV): Cyclopoida (CI-CV); CYC (CVI): Cyclopoida (CVI); HAR: Harpactacoida; NAU CAL: Nauplii Calanus; NAU CIR: Nauplii Cirripede; POEC: Poecilostomatoida. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; ($-$) Excluded variables due to collinearity.

	Muggiaea atlantica (nectophores)		*Muggiaea kochii* (nectophores)		*Muggiaea* spp. gonophores	
	2014	2015	2014	2015	2014	2015
PP						
T						
Sal	0.01***	0.03**	0.02***			0.02**
CAL (CI-CV)	($-$)	($-$)	($-$)	($-$)	($-$)	($-$)
CAL (CVI)						
CYP CIR	0.28**		0.28**		0.31***	
CYC (CI-CV)		0.35**				0.15*
CYC (CVI)						
HAR						
NAU CAL					0.19***	
NAU CIR						
POEC				0.22***		
Zone	($-$)	($-$)	($-$)	($-$)	($-$)	($-$)
F-statistic	20.25	15.73	19.68	13.55	28.68	10.89
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01
R^2	0.52	0.48	0.52	0.28	0.61	0.24

(Z1 and Z2).

The direct influences of local hydrology on *Muggiaea* spp. may be amplified by trophic interactions with the main prey, as confirmed by the significant influence of salinity and prey abundance on the spatial and seasonal distribution of both species. In 2014, abundance of both species nectophores were positively correlated with cirripede cypris, while gonophores increased also with calanoid nauplius. Calanoid copepods are the most common group in the estuary (Marques et al., 2014, 2006) and several studies have shown that copepods (including their several developmental stages) represent the dominant dietary component of *M. atlantica* (Purcell, 1982). Other prey organisms can also be important for the diet of siphonophores, particularly when copepods are scarce (Purcell, 1983, 1981). In the autumn of 2014 we observed an increase of cirripede *cypris* which seem to have represented an important food source for *Muggiaea* spp. during a low copepod abundance period. According to Hansson et al. (2005), cirripedes are captured with high efficiency due to the lack of obvious escape responses, while copepodites can respond to hydrodynamic signals with pronounced escape behaviours (Kiørboe et al., 1999). On the other hand, the relationship of *Muggiaea* spp. nectophores with Cyclopoida copepodites and Poecilostomatoida observed in 2015 reinforces the role of the marine influence, as the species of these Classes are mainly marine (Marques et al., 2014, 2006). This link can be indicative of the food preferences of both *Muggiaea* species. As voracious predators, it has been shown that the distribution of siphonophores strongly depends on the food availability (Greve, 1994; Mills, 1995; Purcell, 1982) and the number of eudoxids produced increases with the amount of food (Dallot et al., 1988; Purcell, 1982).

This work described the distribution patterns of *M. atlantica* and *M. kochii* in the Mondego estuary during two hydrologically distinct years. Salinity was revealed as the key factor influencing their seasonal, spatial and inter-annual variation, along with prey availability. Since data on siphonophores are relatively scarce and the biology of these two species is still poorly known, additional studies should focus on the variables affecting their life cycle (nectophores and eudoxid stages), their distribution in the coastal systems and their disruptive role in marine food webs. It would also be interesting to investigate the rate of gonophore production as a function of temperature and salinity, particularly under a climate change scenario.

Acknowledgements

References

Angeles, A., 1971. Siphonophores of the pacific with a review of the world distribution. Berkeley Plan. J. 26, 14–32.

Azeiteiro, U.M., Marques, J.C., Ré, P., 1999. Zooplankton annual cycle in the Mondego river estuary (Portugal). Museu Bocage.

Baptista, J., Martinho, F., Nyitrai, D., Pardal, M.A., Dolbeth, M., 2015. Long-term functional changes in an estuarine fish assemblage. Mar. Pollut. Bull. 97, 125–134.

Batistić, M., Jasprica, N., Carić, M., Lučić, D., 2007. Annual cycle of the gelatinous invertebrate zooplankton of the eastern South Adriatic coast (NE Mediterranean). J. Plankton Res. 29, 671–686.

Batistić, M., Lučić, D., Carić, M., Garić, R., Licandro, P., Jasprica, N., 2013. Did the alien calycophoran *Muggiaea atlantica* outcompete its native congeneric *M. kochi* in the marine lakes of Mljet Island (Croatia)? Mar. Ecol. 34, 3–13.

Blackett, M., Licandro, P., Coombs, S.H., Lucas, C.H., 2014. Long-term variability of the siphonophores *Muggiaea atlantica* and *M. kochi* in the Western English Channel. Prog. Oceanogr. 128, 1–14.

Blackett, M., Lucas, C.H., Harmer, R., Licandro, P., 2015. Population ecology of *Muggiaea atlantica* (Cnidaria, Siphonophora) in the western English channel. Mar. Ecol. Prog. Ser. 535, 129–144.

Bouillon, J., Medel, M.D., Pagès, F., Gili, J.M., Boero, F., Gravili, C., 2004. Fauna of the mediterranean Hydrozoa. Sci. Mar. 68 (2), 1–449.

Carré, C., Carré, D., 1991. A complete life cycle of the calycophoran siphonophore *Muggiaea kochi* (Will) in the laboratory, under different temperature conditions: ecological implications. Philos. Trans. R. Soc. B Biol. Sci. 334, 27–32.

Chambers, J.M., 1992. Linear models. In: Chambers, J.M., Hastie, T.J. (Eds.), Statistical Models in S. Wadsworth & Brooks/Cole.

Cunningham, J.T., 1892. On a species of siphonophore observed at plymouth. J. Mar. Biol. Assoc. U. K. 2 212-212.

D'Ambrosio, M., Molinero, J.C., Azeiteiro, U.M., Pardal, M.Â., Primo, A.L., Nyitrai, D., Marques, S.C., 2016. Interannual abundance changes of gelatinous carnivore zooplankton unveil climate-driven hydrographic variations in the Iberian Peninsula. Portugal. Mar. Environ. Res. 120, 103–110.

Dallot, S., Goy, J., Carré, C., 1988. Peuplements de carnivores planctoniques gélatineux et structures productives en Méditerranée occidentale. Oceanol. Acta SP 8, 193–209.

Gili, J.M., Pagès, F., Riera, T., 1987. Distribución de las especies más frecuentes de sifonóforos calicóforos en la zona norte del Mediterráneo Occidental. Invest. Pesq. 51, 323–338.

Greve, W., 1994. The 1989 German Bight invasion of *Muggiaea atlantica*. ICES J. Mar. Sci. 51, 355–358.

Guerrero, E., Marrodán, A., Sabatés, A., Orejas, C., Gili, J.-M., 2016. High spatial heterogeneity of two planktonic cnidarian species related to the variability of a shelf-slope front at short time scales. Sci. Mar. 80, 487–497.

Hansson, L.J., Moeslund, O., Kiørboe, T., Riisgård, U., 2005. Clearance rates of jellyfish and their potential predation impact on zooplankton and fish larvae in a neritic ecosystem (Limfjorden, Denmark). Mar. Ecol. Prog. Ser. 304, 117–131.

Kiørboe, T., Saiz, E., Visser, A., 1999. Hydrodynamic signal perception in the copepod *Acartia tonsa*. Mar. Ecol. Prog. Ser. 179, 97–111.

Kirkpatrick, P.A., Pugh, P.R., 1984. Siphonophores and Velellids: Keys and Notes for the Identification of the Species. Brill Archive.

Kršinić, F., Njire, J., 2001. An invasion by *Muggiaea atlantica* Cunningham 1892 in the northern Adriatic Sea in the summer of 1997 and the fate of small copepods. Acta Adriat. 42, 49–59.

Licandro, P., Souissi, S., Ibanez, F., Carré, C., 2012. Long-term variability and environmental preferences of calycophoran siphonophores in the Bay of Villefranche (northwestern Mediterranean). Prog. Oceanogr. 97–100, 152–163.

Lopez-Lopez, L., Molinero, J.C., Tseng, L.-C., Chen, Q.-C., Hwang, J.-S., 2013. Seasonal variability of the gelatinous carnivore zooplankton community in Northern Taiwan. J. Plankton Res. 35, 677–683.

Lucas, C.H., Jones, D.O.B., Hollyhead, C.J., Condon, R.H., Duarte, C.M., Graham, W.M., Robinson, K.L., Pitt, K.A., Schildhauer, M., Regetz, J., 2014. Gelatinous zooplankton biomass in the global oceans: geographic variation and environmental drivers. Global Ecol. Biogeogr. 23, 701–714.

Lynam, C.P., Hay, S.J., Brierley, A.S., 2005. Jellyfish abundance and climatic variation: contrasting responses in oceanographically distinct regions of the North Sea, and possible implications for fisheries. J. Mar. Biol. Assoc. U. K. 85, 435–450.

Mackie, G.O., Pugh, P.R., Purcell, J.E., 1987. Siphonophore biology. Adv. Mar. Biol. 24 97-262.

Mapstone, G.M., 2014. Global diversity and review of siphonophorae (Cnidaria: Hydrozoa). PLoS One 9, e87737.

Marques, S.C., Azeiteiro, U.M., Leandro, S.M., Queiroga, H., Primo, A.L., Martinho, F., Viegas, I., Pardal, M.Â., 2008. Predicting zooplankton response to environmental changes in a temperate estuarine ecosystem. Mar. Biol. 155, 531–541.

Marques, S.C., Azeiteiro, U.M., Marques, J.C., Neto, J.M., Pardal, M.Â., 2006. Zooplankton and ichthyoplankton communities in a temperate estuary: spatial and temporal patterns. J. Plankton Res. 28, 297–312.

Marques, S.C., Primo, A.L., Martinho, F., Azeiteiro, U.M., Pardal, M.Â., 2014. Shifts in estuarine zooplankton variability following extreme climate events: a comparison between drought and regular years. Mar. Ecol. Prog. Ser. 499, 65–76.

Martell-Hernández, L.F., Sánchez-Ramírez, M., Ocaña-Luna, A., 2014. Distribution of planktonic cnidarian assemblages in the southern Gulf of Mexico, during autumn. Rev. Chil. Hist. Nat. 87, 18.

Martinho, F., Leitão, R., Viegas, I., Dolbeth, M., Neto, J.M., Cabral, H.N., Pardal, M.A., 2007. The influence of an extreme drought event in the fish community of a southern Europe temperate estuary. Estuar. Coast Shelf Sci. 75, 537–546.

Mills, C.E., 1995. Medusae, siphonophores, and ctenophores as planktivorous predators in changing global ecosystems. ICES J. Mar. Sci. 52, 575–581.

Mills, C.E., 2001. Jellyfish Blooms: Are Populations Increasing Globally in Response to.

Molinero, J.C., Ibanez, F., Nival, P., Buecher, E., Souissi, S., 2005. The North Atlantic climate and the northwestern Mediterranean plankton variability. Limnol. Oceanogr. 50, 1213–1220.

Nagata, R.M., Nogueira Júnior, M., Brandini, F.P., Haddad, M.A., 2014. Spatial and temporal variation of planktonic cnidarian density in subtropical waters of the Southern Brazilian Bight. J. Mar. Biol. Assoc. U. K. 1387–1400.

Palma, S., Silva, N., 2006. Epipelagic siphonophore assemblages associated with water masses along a transect between Chile and Easter Island (eastern South Pacific Ocean). J. Plankton Res. 28, 1143–1151.

Pavez, M.A., Landaeta, M.F., Castro, L.R., Schneider, W., 2010. Distribution of carnivorous gelatinous zooplankton in the upwelling zone off central Chile (austral spring 2001). J. Plankton Res. 32, 1051–1065.

Pereira, R., Teodósio Chíaro, M.A., Garrido, S., 2014. An experimental study of *Aurelia aurita* feeding behaviour : inference of the potential predation impact on a temperate estuarine nursery area. Estuar. Coast Shelf Sci. 146, 102–110.

Pitt, K. a, Clement, A.-L., Connolly, R.M., Thibault-Botha, D., 2008. Predation by jellyfish on large and emergent zooplankton: implications for benthic-pelagic coupling. Estuar. Coast Shelf Sci. 76, 827–833.

Primo, A.L., Azeiteiro, U.M., Marques, S.C., Martinho, F., Pardal, M.Â., 2009. Changes in zooplankton diversity and distribution pattern under varying precipitation regimes in a southern temperate estuary. Estuar. Coast Shelf Sci. 82, 341–347.

Primo, A.L., Azeiteiro, U.M., Marques, S.C., Pardal, M.Â., 2011. Impact of climate variability on ichthyoplankton communities: an example of a small temperate estuary. Estuar. Coast Shelf Sci. 91, 484–491.

Primo, A.L., Kimmel, D., Marques, S.C., Martinho, F., Azeiteiro, U., Pardal, M.A., 2015. Zooplankton community responses to regional-scale weather variability: a synoptic climatology approach. Clim. Res. 62, 189–198.

Primo, A.L., Marques, S.C., Falcão, J., Crespo, D., Pardal, M.Â., Azeiteiro, U.M., 2012. Environmental forcing on jellyfish communities in a small temperate estuary. Mar. Environ. Res. 79, 152–159.

Pugh, P.R., 1974. The vertical distribution of the siphonophores collected during the Sond Cruise, 1965. J. Mar. Biol. Assoc. U. K. 54, 25–90.

Pugh, P.R., 1989. Gelatinous zooplankton - the forgotten fauna. Prog. Underw. Sci. 14, 67–78.

Purcell, J.E., 1981. Dietary composition and diel feeding patterns of epipelagic siphonophores. Mar. Biol. 65, 83–90.

Purcell, J.E., 1982. Feeding and growth of the siphonophore *Muggiaea atlantica*. J. Exp. Mar. Biol. Ecol. 62, 39–54.

Purcell, J.E., 1983. Digestion rates and assimilation efficiencies of siphonophores fed zooplankton prey. Mar. Biol. 73, 257–261.

Purcell, J.E., 2005. Climate effects on formation of jellyfish and ctenophore blooms: a review. J. Mar. Biol. Assoc. U. K. 85, 461–476.

Purcell, J.E., 2012. Jellyfish and ctenophore blooms coincide with human proliferations and environmental perturbations. Ann. Rev. Mar. Sci. 4, 209–235.

R Core Team, 2014. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria 2013.

Robinson, K.L., Graham, W.M., 2013. Long-term change in the abundances of northern Gulf of Mexico scyphomedusae *Chrysaora* sp. and *Aurelia* spp. with links to climate variability. Limnol. Oceanogr. 58, 235–253.

Sabatés, A., Pagès, F., Atienza, D., Fuentes, V., Purcell, J.E., Gili, J.M., 2010. Planktonic cnidarian distribution and feeding of *Pelagia noctiluca* in the NW Mediterranean Sea. Hydrobiologia 645, 153–165.

Sanvicente-Añorve, L., Alba, C., Flores-Coto, C., Castillo-Rivera, M., 2009. Siphonophores off a riverine system in the southern Gulf of Mexico: factors affecting their distribution and spatial niche breadth and overlap. Aquat. Ecol. 43, 423–435.

Will, J.G.F., 1844. Horae Tergestinae oder Beschreibung und Anatomie der im Herbste 1843 bei Triest beobachteten Akalephen, Leipzig Wilson.

Zakaria, H.Y., 2004. Pelagic coelenterates in the waters of the western part of the Egyptian Mediterranean Coast during summer and winter. Oceanologia 46.