



Contents lists available at [ScienceDirect](#)

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss



Abundance, seasonal patterns and diet of the non-native jellyfish *Blackfordia virginica* in a Portuguese estuary



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ARTICLE INFO

Article history:

Received 21 October 2014

Received in revised form

15 July 2015

Accepted 25 July 2015

Available online 27 July 2015

Keywords:

Alien species

Hydrozoa

Jellyfish blooms

Mira estuary

Predation impact

Trophic ecology

ABSTRACT

Blackfordia virginica, a non-indigenous hydrozoan introduced in many systems around the world, has been observed in the Mira estuary, southwest of Portugal, since 1984. Monthly sampling (January 2013–January 2014) at a fixed location with high abundance of the medusae confirmed the occurrence of a seasonal cycle associated with temperature and photoperiod. The beginning of the medusa cycle occurred in May immediately after the spring zooplankton bloom during April. Examination of the gut contents of *B. virginica* medusae revealed that copepods, the most abundant group in the zooplankton community, were highly predated. Barnacle nauplii, decapod crustacean larvae and anchovy eggs were also identified in the guts. The medusae showed positive selection for copepods, and negative selection for barnacle nauplii, decapod crustacean larvae and anchovy eggs. The mortality rate of copepods (used as a model prey group) induced by medusae predation was estimated and showed the potential impact of this species in the ecosystem, ranging between 2.34 d^{-1} and 0.02 d^{-1} , with a minimum copepod half-life of 0.30 days.

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1. Introduction

Blackfordia virginica is a hydrozoan which presents a benthic polyp and a planktonic medusa with separate sexes (Mills and Sommer, 1995). Its capacity to colonize brackish water systems with a wide range of temperatures and salinities indicates a high potential for dispersal (Moore, 1987; Bardi and Marques, 2009). *B. virginica* has been introduced worldwide and some authors suggest that ballast water exchanges and hull fouling might be the main vectors for *B. virginica* transport between systems (Zaitsev and Oztürk, 2001; Golemansky, 2007). The origin of this species is controversial: some authors indicate it is endemic to the Atlantic coast of North America (Zaitsev and Ozturk, 2001), where the medusa was first described in 1910 (Mayer, 1910), while others state it is native to the Black Sea (Mills and Sommer, 1995; Graham and Bayha, 2007). Certain is that this species is present from South America (Álvarez-Silva et al., 2003; Genzano et al., 2006; Nogueira Jr. and De Oliveira, 2006; Bardi and Marques, 2009) to the west coast of North America (Mills and Rees, 2000; Wintzer et al., 2013),

and also in Asia (Zhang, 1982; Santhakumari et al., 1997). In Europe, this species is present in the western coast of France (Denayer, 1973) and in Portugal, namely in the Mira estuary, where it was first observed in 1984 (Moore, 1987), and has been reported from more recent work conducted in the estuary (Ré, 1987, 1996; Mattos, 1995). Nearly twenty years later it was also detected in southern Portugal in the Guadiana estuary (Chícharo et al., 2009), but in this system the medusae have not been recorded every year (Alexandra Teodósio, CCMAR, Univ. Algarve, Portugal, pers. comm.).

The occurrence of the *B. virginica* medusa is seasonal in many estuaries, with peak abundances during the warmer periods of the year (Bardi and Marques, 2009; Wintzer et al., 2013). The medusa is thought to be a non-selective zooplankton predator which feeds primarily on adult and naupliar copepods (Mills and Sommer, 1995; Wintzer et al., 2013). Moreover, Wintzer et al. (2013) found higher prey consumption during the periods when copepod nauplii were more abundant. However, its predation impact on the zooplankton community is poorly known.

To address the gaps in knowledge of the *B. virginica* medusa population in the Mira estuary, the present work aimed to (1) study the medusa seasonal occurrence in the estuary, by determining the population structure (density and size structure); and (2) examine its potential predation impact on the zooplankton community.

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2. Materials and methods

2.1. Study area

The Mira estuary ($37^{\circ} 40'N$; $8^{\circ} 45'W$) is a relatively small tidal estuary located in the southwestern coast of Portugal. The estuary is approximately 40 km long and 400 m wide near its mouth (Blanton et al., 2000). The mean depths are 1.2 m upstream and 8 m near the river mouth and the residence time is fourteen days (Costa, 2004).

The Mira estuary is considered the least threatened, since the level of anthropogenic pressure is reduced and much lower than in other Portuguese estuaries (Bettencourt et al., 2004). It is less exposed to nutrient and chemical pollution since there are no large urban and industrial areas, however, there are some intensive agriculture units, cattle breeding, aquaculture activities and domestic sewage discharge (Castro and Freitas, 2006). The freshwater flow regime was also changed by the construction of an agriculture reservoir in 1967 (Santa Clara), located 50 km upstream of the river mouth.

To follow the seasonal cycle of the *B. virginica* medusa in the estuary, a location with previously-documented high abundances (Marques, 2013) was selected. This sampling site is located approximately 14.5 km from the sea ($37^{\circ} 39.756'N$; $8^{\circ} 43.256'W$) (Fig. 1).

$$\text{Occurrence index(OI)} = (\text{number of gastrovascular cavities containing prey } i / \text{number of full gastrovascular cavities}) \times 100$$

$$\text{Numerical index(NI)} = (\text{number of prey } i \text{ in the gastrovascular cavities} / \text{total prey number in gastrovascular cavities}) \times 100$$

$$\text{Vacuity index(VI)} = (\text{number of empty gastrovascular cavities} / \text{total number of observed gastrovascular cavities}) \times 100$$

2.2. Sampling

B. virginica medusa and other zooplankton sampling was conducted monthly between January 2013 and January 2014 to determine the seasonal occurrence of the medusa. During the initial period of the medusa seasonal cycle (from April until June) weekly samples were collected in order to determine more precisely the beginning of the medusa stage in the estuary. Medusae and zooplankton were collected by boat at 0.5 m depth by horizontal hauls (2 replicates) of a zooplankton net (200 µm mesh, 1.5 m length, 0.4 m mouth diameter) fitted with a flowmeter. All trawls were five minutes in duration and samples were always collected 3 h after low-tide and immediately preserved in a buffered 4% formaldehyde solution. In addition to plankton sampling, environmental variables (temperature, dissolved oxygen concentration and salinity) were measured using a multiparameter probe.

2.3. Laboratory work

In the laboratory, *B. virginica* medusae were separated from the other zooplankton taxa using different mesh size sieves. The total volume of medusae and zooplankton was measured for each sample.

Medusa density (individuals/m³) was calculated based on counts of the total number of individuals per sample or in a 300 ml subsample of medusae biovolume, when samples exceeded that volume. Subsamples of 60 ml were used to measure the medusae bell diameter under a stereomicroscope (maximum 50×

magnification). Mean medusa population densities for each sampling date were estimated based on the replicate densities. Specimens were dissected for identification of zooplankton prey found in their gastrovascular cavities.

In order to calculate the density (individuals/m³) of the remaining zooplankton, a sample volume containing a minimum of 300 individuals was subsampled, using a Stempel pipette's similar method. The organisms were counted and identified to the lowest practical taxon under a stereomicroscope using zooplankton taxonomic resources (Trégouboff and Rose, 1957; Smith DeBoyd and Johnson, 1996; Johnson and Allen, 2012).

2.4. Data analysis

2.4.1. Medusae abundance and environmental variables

To determine the environmental variables that influenced medusae density over time in the Mira estuary, a nonparametric Spearman's ρ rank correlation test was performed using temperature, dissolved oxygen, salinity and photoperiod ($p < 0.01$).

2.4.2. Diet and predation impact

To quantify the zooplankton prey composition and feeding activity of *B. virginica* in the Mira estuary, the following indices were calculated following Hyslop (1980):

Medusa feeding selectivity was examined using the Jacobs selectivity index (D) (Jacobs, 1974) using the formula:

$$D = (C_i - A_i) / (C_i + A_i - 2C_iA_i),$$

where C_i and A_i are the relative frequency of prey i in the guts and in the environment, respectively. The Jacobs index varies from -1 to 0 for negative selection; and from 0 to 1 for positive selection. Zero values suggest that a prey is used in proportion to its availability.

Predation impact, μ , of the medusae (i.e., the medusa population clearance rate, volume filtered by the jellyfish population in one m³ water column per day = m³ m⁻³ d⁻¹) on the copepod population (the main prey item of *B. virginica* medusae and therefore used as prey model), was estimated in each sampling date when gut contents were present, and was calculated following Hansson et al. (2005):

$$\mu = F_i D_i,$$

where D_i is the density of medusa of species i and F_i is the medusa individual clearance rate.

Clearance rates can be used to measure the predation rate per volume of water per unit of time (Larson, 1991). In the present study, the clearance rate was estimated indirectly following the expression:

$$I = G/E,$$

where I is prey ingestion rate, G is the number of prey in the guts

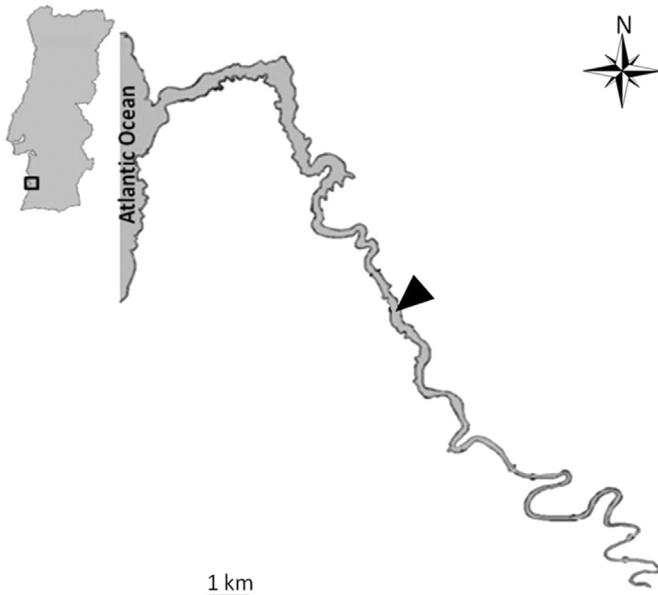


Fig. 1. Location of the fixed sampling site established in the Mira estuary ($37^{\circ}39.756'N$; $8^{\circ}3.256'W$). Adapted from Google Maps.

and E is the prey digestion time. And F_i was calculated as:

$$F_i = I/C,$$

where C is the prey concentration in the environment (Hansson et al., 2005).

Copepod digestion times used in this study were estimated in the laboratory during experiments conducted by Alpa Wintzer, UC Davis, California (pers. comm.) using *Eurytemora affinis* as prey. The experiment was performed in filtered saltwater (salinity of 16 and temperature of $21^{\circ}C$) using *B. virginica* medusae with 7.69 ± 0.33 mm bell diameter. The mean number of copepod in the guts and the copepod density in the environment were estimated from the sampled replicates.

The theoretical half-life ($t_{1/2}$) of copepods, the time required to reduce a prey organism (copepod) concentration by 50% (Hansson et al., 2005), was also calculated as:

$$t_{1/2} = \ln 2/\mu.$$

Additionally, a nonparametric Spearman's ρ rank correlation test was used to examine the relationship between the number of ingested prey organisms by the medusae and the concentration of prey in the environment ($p < 0.01$).

3. Results

3.1. Medusa density and population structure

During 2013, *B. virginica* medusae showed a seasonal occurrence in the Mira estuary (Fig. 2). In 2013, medusae were first collected in low numbers in early May, and two peaks of density occurred in the end of June (64.78 ind./m 3) and in September (92.41 ind./m 3). Following the maximum density in September, abundance declined gradually through December (0.17 ind./m 3), and no medusae were observed in January 2014.

Medusae bell diameter varied along time: small individuals were observed during different sampling occasions, and towards the end of the medusae seasonal occurrence mainly large individuals were collected (Fig. 3). The minimum bell size recorded

was $238\text{ }\mu\text{m}$ in June and the maximum bell size was 20 mm in November. The proportion of very small medusae (bell diameter ≤ 1 mm) was highest in May and June.

3.2. Zooplankton density and composition

The highest densities of zooplankton occurred in spring (namely in April and May), while the lowest densities of zooplankton occurred during winter months and early spring (February – beginning of April), when medusae were still not present (Figs. 2 and 4). The peak density of zooplankton occurred just prior to the onset of the *B. virginica* bloom, on April 26.

The zooplankton community consisted of 17 major groups (Fig. 4). The highest taxa diversity occurred in April and June, while the lowest taxa diversity occurred during the coldest months (January 2013–March 2013 and October 2013–January 2014). Copepods were the most abundant zooplankton during the study period. From winter to early spring (January–April 2013), cladocerans, decapod crustacean larvae and isopods were the most abundant groups after copepods. During spring-summer (end of April until August) many meroplanktonic invertebrates were also present: barnacle nauplii and cyprids were very abundant followed by gastropods and decapod crustacean larvae. Eggs and larvae of the European anchovy *Engraulis encrasicolus* were also abundant from May to June. During the autumn – winter period (October 2013 to January 2014), isopods were the most abundant zooplankton, after copepods (Fig. 4).

3.3. Medusae occurrence and environmental variables

Environmental conditions showed a seasonal variation, with lower water temperatures and lower salinities occurring during winter months (January–April 2013 and November 2013–January 2014) and higher temperatures and salinities occurring between May and October 2013. The maximum temperature and salinity recorded were approximately $25^{\circ}C$ and 27 , respectively, in August. Dissolved oxygen levels were generally higher during winter and decreased during summer months (maximum of 9.62 mg/L in March, and minimum of 1.83 mg/L in July).

Significant correlations were found between *B. virginica* medusa density and water temperature ($\rho = 0.781$; $P < 0.01$), photoperiod ($\rho = 0.478$; $P = 0.024$), percentage of dissolved oxygen ($\rho = -0.512$; $P = 0.035$) and salinity ($\rho = 0.810$; $P < 0.001$). Medusae were first observed in the estuary when water temperature reached $18^{\circ}C$ (air temperature $15.7^{\circ}C$) and photoperiod was approximately 13.9 h. The medusa seasonal bloom occurred with a temperature range of $12^{\circ}C$ – $23.55^{\circ}C$, a dissolved oxygen range of 1.83 – 8.25 mg/L, a salinity range of 12.00 – 27.52 and a number of daylight hours higher than 9.1 . The last collection of the medusae occurred in the beginning of the winter (mid December), when temperature and photoperiod were lowest ($11.99^{\circ}C$; 9.1 h) (Fig. 2).

3.4. Medusae feeding

Of the 5958 guts examined, only 633 contained zooplankton prey (10.6%). The vacuity index was generally high even for sampling dates with recorded gut contents (Table 1). The highest mean number of prey found in the guts was recorded for December 12 (5.75 ± 1.89 prey items), while the lowest was observed for September 10 (0.01 ± 0.01 prey items). The number of prey found in the medusae guts ranged from 0 to 13 and no significant correlation was found between the number of prey items present in the medusae guts and prey density in the environment ($p > 0.01$). The medusae with identifiable zooplankton

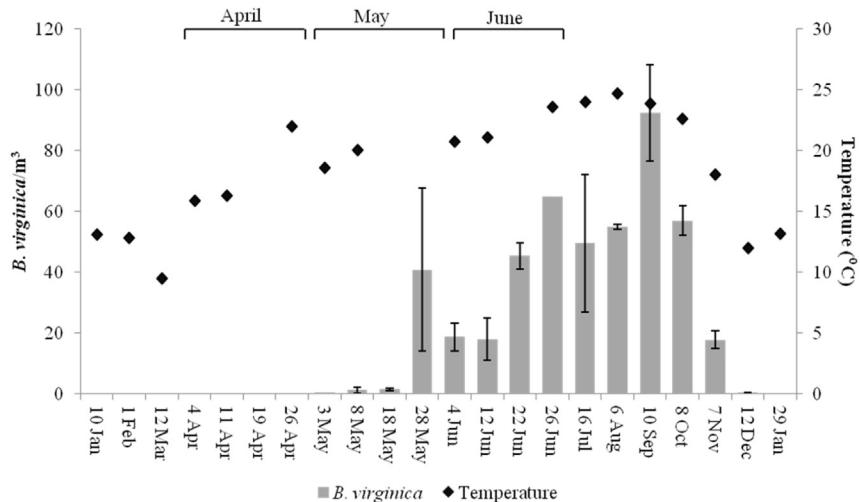


Fig. 2. *B. virginica* medusa density (\pm SE) and water temperature ($^{\circ}$ C) at the fixed sampling site in the Mira estuary. Samples were collected weekly from April to June and monthly in other months.

prey in the guts presented a bell diameter ranging from 1 mm to 16 mm (mean 7.44 ± 0.13 mm). Copepods (mainly copepodite stages of *Acartia* spp.) were the most abundant prey found in the medusae guts (Table 2). Barnacle nauplii, decapod crustacean larvae and anchovy eggs were also preyed upon, but they were much less abundant in the medusa's diet. Jacobs selectivity index showed positive selection for copepods in all samples (Table 3). Barnacle nauplii, decapod crustacean larvae and anchovy eggs were negatively selected.

3.5. Medusa predation impact

The predation impact of medusae on the copepod population and copepod half-life were not homogeneous, as the vacuity index and, especially, the medusae and copepods densities fluctuated throughout the sampling period. The predation impact ranged from 0.02 d^{-1} , on September 10, to 2.34 d^{-1} , on June 4 (Table 4). Accordingly, the theoretical half-life of copepods was lowest on June 4, when the medusa predation reduced the copepod concentration by 50% in 0.30 days, while the highest copepod half-life was found on September 10, with 41.23 days.

4. Discussion

4.1. Seasonal occurrence and population structure

The present study aimed at reporting for the first time the seasonal cycle of *B. virginica* medusa in the Mira estuary and in Portugal. Moore (1987) indicated the presence of the medusa stage in the Mira estuary at least from April to June and also in September and November in 1984, when it was first reported. Previous surveys carried out in the Mira estuary, namely those addressing ichthyoplankton and copepod distribution, indicated that the medusa was present at least from July until October (Mattos, 1995) and that its high abundance caused zooplankton net clogging during summer months (Ré, 1987, 1996). The high densities and the continuous presence of medusae reported informally for other types of surveys such as those referenced above (Ré, 1987, 1996; Mattos, 1995) showed the occurrence of a well established population of *B. virginica* in the Mira estuary.

The present study confirmed the seasonal occurrence of the *B. virginica* medusa in the Mira estuary, as observed in other systems worldwide (Santhakumari et al., 1997; Bardi and Marques, 2009; Wintzer et al., 2013). *B. virginica* medusae occurred from May through December, and its abundance was positively correlated with temperature, photoperiod and salinity, and negatively influenced by dissolved oxygen. Temperature, photoperiod, salinity and dissolved oxygen are likely to be the triggering parameters which determine the medusa cycle. Medusae presence and abundance depend on the presence and activity of benthic stages (polyps), which asexually produce medusae when environmental conditions are favorable (Purcell, 1985; Brewer and Feingold, 1991; Lucas, 2001; Boero et al., 2008). Polyp presence is continuous in the benthos in the form of resting hydrorhizae or actively feeding colonies that will generate the next medusa cycle (Boero et al., 2008). In temperate regions, the environmental and biological factors that induce medusae release by polyps are known for only a few jellyfish species (Lucas, 2001; Purcell, 2005), but it appears to be a consequence of a synergistic effect of temperature, light, food and salinity (Lucas, 2001; Ma and Purcell, 2005; Wintzer et al., 2011a).

B. virginica medusa occurrence was also seasonal in Napa and Petaluma Rivers, San Francisco estuary, California, with medusa found during late spring and summer (Mills and Sommer, 1995;

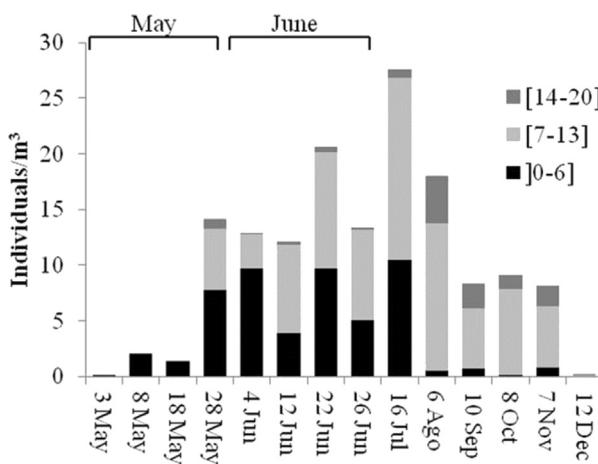


Fig. 3. Bell diameter (mm) of *B. virginica* medusae recorded at the fixed sampling site in the Mira estuary. Samples were collected weekly from April to June and monthly in other months.

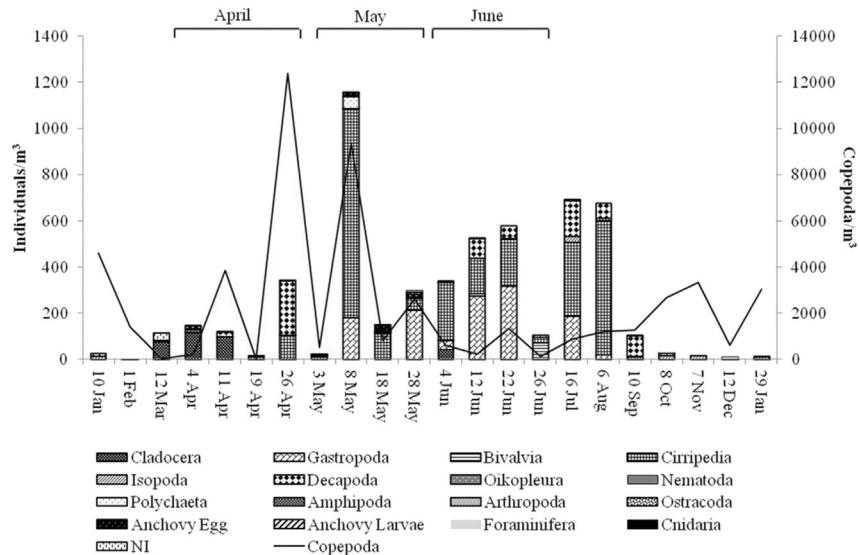


Fig. 4. Zooplankton community density and composition at the fixed sampling site in the Mira estuary. Samples were collected weekly from April to June and monthly in other months.

(Wintzer et al., 2013). In Napa and Petaluma Rivers, medusae were present with a temperature range of 16.5–23 °C, a salinity interval of 10.1–20.6 and dissolved oxygen values between of 3.8–6.9 mg/L (Wintzer et al., 2013), a narrower interval for these environmental variables in the US systems compared to the Mira estuary (present study).

The onset of the planktonic phase for *B. virginica* in the studied area occurred after the zooplankton bloom in spring. In temperate regions there is a seasonal variation of primary and secondary production, with typically higher zooplankton abundances in spring-summer and with a secondary peak in autumn in some systems (Raymont, 1983). Accordingly, the zooplankton bloom in the Mira estuary followed the typical pattern: higher densities in spring (April and May) and in autumn (October and November) and

lower densities during winter and summer months. The beginning of the medusa cycle followed the first zooplankton bloom and ended when an abrupt decrease in the zooplankton density was observed. This suggests that the food availability may also affect the medusae production by the hydroid in the Mira estuary. Ma and Purcell (2005), assessing the effects of environmental conditions (temperature, salinity and food consumption) on polyp and medusa bud production of the hydrozoan *Moerisia lyonsi*, found that higher food consumption, combined with other environmental factors (such as temperature and salinity), promoted the production of the planktonic medusae and increased the medusae bud production by polyps.

Medusa release appeared to be more intense between May and July and in November, towards the end of the cycle, as indicated by

Table 1

Vacuity index, range of prey items in the guts and mean zooplankton prey abundance for sampling dates when medusa gut contents were present.

Sampling date	Vacuity index	Range of prey items	Mean prey nr. (SE)
28 May	96.7	0–7	0.07 (0.01)
4 June	46.7	0–12	1.13 (0.07)
12 June	88.2	0–6	0.17 (0.02)
10 September	99.7	0–1	0.01 (0.00)
8 October	95.9	0–8	0.10 (0.03)
7 November	70.4	0–13	1.16 (0.13)
12 December	25.0	0–13	5.75 (1.89)

Table 3

B. virginica medusae zooplankton prey selectivity estimated by Jacobs selectivity index (*D*) for sampling dates when gut contents were present. *D* > 0 – positive selection; *D* < 0 – negative selection.

Sampling date	Copepod	Barnacle Nauplius	Decapod Larvae	Anchovy egg
28 May	0.99	—	—	-0.89
4 June	0.95	-0.93	-0.95	-0.96
12 June	0.99	-0.89	-0.78	—
10 September	1.00	—	—	—
8 October	1.00	—	—	—
7 November	1.00	—	—	—
12 December	1.00	—	—	—

Table 2

Zooplankton prey occurrence index (OI) and numerical index (NI) in *B. virginica* medusae guts for sampling dates when gut contents were present.

Sampling date	Copepod		Barnacle Nauplius		Decapod Larvae		Anchovy egg	
	OI	NI	OI	NI	OI	NI	OI	NI
28 May	97.8	97.8	—	—	—	—	2.2	2.2
4 June	100.0	98.7	2.9	1.1	0.6	0.1	0.3	0.1
12 June	100.0	99.3	1.1	0.3	1.1	0.4	—	—
10 September	100.0	100.0	—	—	—	—	—	—
8 October	100.0	100.0	—	—	—	—	—	—
7 November	100.0	100.0	—	—	—	—	—	—
12 December	100.0	100.0	—	—	—	—	—	—

Table 4

B. virginica medusae predation impact on the copepod population (μ , days⁻¹) and copepod half-life ($t_{1/2}$, days), considering sampling dates when gut contents were present.

Sampling date	μ	$t_{1/2}$
28 May	0.06	11.43
4 June	2.34	0.30
12 June	0.56	1.22
10 September	0.02	41.23
8 October	0.07	10.33
7 November	0.24	2.84
12 December	0.06	10.92

the collection of very small medusae on those sampling dates. Concomitantly, the bell diameter increased over the course of the medusae cycle, with only larger individuals observed at the end. However, the average bell size in Napa and Petaluma rivers, in the early cycle, was ca. 5 mm, and it decreased over the bloom (Wintzer et al., 2013). In the final period, the size classes represented were between 1 mm and 2 mm (Wintzer et al., 2013). Maximum bell size previously recorded for the Mira estuary was 22.2 mm (Moore, 1987), and in the present study the largest bell diameter was 20.0 mm. In the Guadiana estuary, Chicharo et al. (2009) found specimens ranging from 6 to 19 mm. The bell size diameter registered in the Portuguese estuaries is therefore higher than the 10 mm measured in other estuaries (Mills and Sommer, 1995; Genzano et al., 2006).

A maximum *B. virginica* medusa density of 92.4 ind./m³ was estimated in this study, while previous reports indicate a density of 66.6 ind./m³ in the Mira estuary (Moore, 1987; mesh size not referred by the author). Unpublished data from a parallel study to the present one found a density of 1689.3 medusae/m³ in the Mira estuary upstream from the present study sampling site (mesh size 200 µm). In other systems, the highest reported abundances varied from 232 ind./m³ in Petaluma river (mesh size 64 µm), San Francisco estuary (Wintzer et al., 2013); 42 ind./m³ in the Bombay Harbor-Thana and Bassein Creek estuarine complex in India (mesh size 330 µm) (Santhakumari et al. 1997); 31.7 ind./m³ in Guadiana Estuary (mesh size 200 µm) (Chicharo et al., 2009); 29.5 ind./m³ in Río de la Plata estuary, Argentina–Uruguay (mesh size not referred by the authors) (Genzano et al. 2006); 10.6 ind./m³ in Babitonga estuary, Brazil (mesh size 300 µm) (Bardi and Marques, 2009) and 2.7×10^{-2} to 2.5×10^{-2} ind./m³ in Antonina Bay, Brazil (mesh size 500 µm) (Nogueira Jr. & De Oliveira, 2006).

Direct comparison of densities among these studies would be impractical as different or unknown mesh sizes were used to collect medusae. However, the medusa abundance estimated in the present study was considerably higher than that indicated by Chicharo et al. (2009) in the Guadiana estuary, with both studies conducted using nets with similar mesh size.

4.2. Feeding ecology

Pelagic cnidarians are usually known as zooplankton predators (Larson, 1991; Mills, 1995) and therefore may exert pressure on zooplankton populations (Olesen, 1995; Hanson et al., 2005; Riisgård et al., 2012), compete with other zooplanktivorous species, including fishes (Purcell and Arai, 2001) and have a direct impact on fish stocks by feeding on fish larvae and eggs (Purcell, 1985). In the present study, the majority of *B. virginica* medusae did not have zooplankton prey in the guts (89.4% vacuity index). Mills and Sommer (1995) also registered a high vacuity index of 85.5% in preserved specimens of *B. virginica* medusae from Napa River. Those authors noted that the high vacuity index may be due to evacuation of prey by medusae caused by late preservation of some individuals in the sample. In contrast, in the Petaluma River the vacuity index was only 5.6% in a diel feeding study (Alpa Wintzer, UC Davis, California, pers. comm.). The high vacuity index found in the present study may indicate loss of gut contents during medusa collection and handling (Larson, 1987), non-continuous feeding, different feeding rates throughout the day, rapid prey digestion or, more likely, medusae feeding on other prey types or materials, such as suspended particles of organic matter, which are very abundant in the Mira estuary. There is some evidence that hydromedusae do not feed continuously, since the indigestible materials must be ejected from the mouth, interrupting the process of food ingestion (Mills, 1981; Migletta et al., 2000). Moreover, Wintzer et al. (2011b) found that seeds, faecal pellets, terrestrial

insects and filamentous algae were also consumed by the hydro-medusa *Maeotias marginata*, although in small amounts. *B. virginica* medusae collected in the Mira and Guadiana estuaries may also feed on diatoms, macroalgae and ciliates, in addition to copepod nauplii, cladocerans, decapod crustacean larvae and early life stages of fishes (Morais et al., 2015). Concerning other hydromedusa species, such as *Rathkea octopunctata* and *Tiaropsis multicirrata*, the scarcity of metazoan prey items may, in fact, be an indication of medusae also feeding on dissolved organic matter, bacteria and nannoplankton (Zelickman et al., 1969). Considering that the predator:prey size ratio may play an important role in food selectivity (Hansen et al., 1994), the consumption of protistan and other prey types (such as microplankton) and materials, especially by the smaller individuals of *B. virginica*, may occur frequently.

The importance of zooplankton prey in the medusae diet may be related, among other factors, to the zooplankton density in the environment, which could change the probability of prey-predator encounter, as jellyfishes are usually non-visual predators. Purcell (1992) found higher copepod densities in Chesapeake Bay, Eastern Coast of North America (mean copepod density 9841 ± 9484 ind./m³), than those found in the Mira estuary (mean copepod density 1793.8 ± 626.8 ind./m³). In Chesapeake Bay all the sampled medusae (scyphomedusa *Chrysaora quinquecirrha*) had zooplankton prey in their gastrovascular cavities (Jennifer Purcell, Western Washington University, pers. comm.). However, *B. virginica* medusae are smaller and different in morphometry from the scyphomedusa studied by Purcell (1992), resulting in a distinct efficiency in capturing the zooplankton prey. Nevertheless, a lower density of copepods may promote the consumption of more vulnerable or more abundant prey types and materials other than zooplankton prey.

A study on the diel feeding of *B. virginica* medusae reported higher prey number in medusae collected during the morning, justified by an increasing prey concentration observed during the morning hours (Wintzer et al., 2013). Purcell (1992) also found a positive relation between prey number in the guts and concentration of prey in the environment for the scyphomedusa *Chrysaora quinquecirrha*. However, in the present study, there was no relationship between the number of prey found in the medusae guts and prey densities in the environment.

The zooplankton diet of *B. virginica* medusae in the Mira estuary consisted mainly of copepodite stages of copepods, similarly to what was found in previous studies for this species (Mills and Sommer, 1995; Wintzer et al., 2013). The most abundant prey items in medusae guts from the Petaluma River were copepod nauplii, cyclopoid copepods and mysids, among others, (Wintzer et al., 2013), while copepods, copepod nauplii and barnacle nauplii were the dominant prey items in the Napa River (Mills and Sommer, 1995). In the present study, *B. virginica* medusa showed positive selectivity for copepods and negative selectivity for barnacle nauplii, decapod crustacean larvae and anchovy eggs. However, the work by Wintzer et al. (2013) found no prey selectivity in Petaluma River. The Jacobs selectivity index does not account for post-encounter factors, such as handling time, digestion rate (Madin, 1988) or prey rejection. Thus, the present results may only be interpreted using factors that precede prey digestion, such as swimming pattern and behavior of medusae and prey in the water column (Mills, 1981; Larson, 1987; Colin et al., 2003), tentacle density and spacing, prey size, type and velocity, and prey post-encounter escape behavior (Mills, 1981; Larson, 1987; Madin, 1988; Spadinger and Maier, 1999). Selectivity is highly variable between sites and time of year, as the composition of the zooplankton community varies and the selectivity indices are very sensitive to changes in zooplankton community structure (Purcell, 1992; Graham and Kroutil, 2001). In the Mira estuary, *B. virginica*

medusae seemed to feed on the most abundant prey item in the zooplankton community. Mills and Sommer (1995) noted that *B. virginica* medusae are quiescent in the water column, a behavior associated with ambush predation (Mills, 1981; Purcell, 1985; Miglietta et al., 2000), where predators capture their prey passively while drifting in the water column and by generating flow fields that entrain prey (Colin et al., 2005). Ambush predators are prone to capture the most abundant and fast swimming prey, like adult copepods and copepodites (Purcell, 1997; Suchman and Sullivan, 1998), increasing the predator-prey encounter rate. Spadiner and Maier (1999) found that selectivity of the freshwater jellyfish *Craspedacusta sowerbii* was closely related to prey size and the most available prey in the environment, the cladoceran *Ceriodaphnia reticulata*, was highly consumed, but it was not the preferential prey. A notable result arose from a study on *Aglaura hemistoma*, an ambush predator, which showed that motionless prey were neglected compared to more motile plankters (Colin et al., 2005). Similarly, the variety of prey captured and consumed by *B. virginica* medusae in the present study may suggest that the most abundant, larger and faster moving prey (like copepods) are preferred over motionless prey, such as anchovy eggs, or smaller prey (Spadiner and Maier, 1999). Selectivity is also related to the type of nematocysts present in the medusae tentacles (Madin, 1988; Regula et al. 2009). Although *B. virginica* medusae tentacles bear microbasic mastigophores (Mills and Sommer, 1995), which can penetrate soft (anchovy eggs) and hard bodied prey (crustacean exoskeleton) (Purcell and Mills, 1988; present study), the higher occurrence of copepods in the medusae guts and their relative preference may suggest that medusae capture copepods more efficiently (faster moving prey, higher encounter probability, as referenced above) than anchovy eggs; or that anchovy eggs may be digested more rapidly, due to the absence of a hard exoskeleton.

4.3. Predation impact

In the present study, the medusae predation impact (the population clearance rate) on the copepod population was calculated based on individual clearance rates obtained theoretically. When the predation impact was highest and the half-life of copepods was lowest (June 4 and 12), the copepod abundance was slightly lower compared with other dates in the same sampling period (summer). Additionally, considering that the copepod *Acartia tonsa* has a generation time ranging between 21 and 34 days (Raymont and Miller, 1962), the half-life obtained on June 4 and 12, 0.30 and 1.12 days, respectively, may suggest that the medusa predation may have a significant impact on the copepod population.

Hansson et al. (2005), in Limfjorden, Denmark, found a minimum copepodite half-life of 8 and 14 days induced by *Aurelia aurita* predation. Comparing those results, the predation impact of *B. virginica* appears to be higher, as the minimum copepod half-life obtained in the present study are lower than those estimated in Limfjorden. Also in Denmark, in Kertinge Nor, *A. aurita* predation impact ranged between 0.01 and 3.51 d⁻¹, with expected copepod half-life of >72 days and 0.20, respectively (Olesen, 1995), which are similar to those found in the present study. The predation impact of scyphomedusae is not, however, fully comparable with the results of the present study, as hydromedusae and scyphomedusae are very different in morphometry. A study on hydromedusa *Obelia geniculata* feeding rates showed a maximum predation impact of >1 d⁻¹ on the copepod *Paracalanus indicus* (Fulton and Wear, 1985), though no information on the copepod half-life was given by the authors.

For predation impact and copepod half-life calculations, the feeding (clearance rate) was assumed to be constant in a 24-h period. However, predation impact may vary as a result of a

putative non-continuous feeding rate of medusae due to changes in zooplankton density (natural patchiness of zooplankton distribution), which interferes with prey availability to predation. Medusae may also feed on other prey types and on organic matter available in the water column, thus reducing the pressure on the copepod population.

Chícharo et al. (2009) noted a reduced abundance of eggs of the European anchovy *E. encrasicolus* at locations where *B. virginica* was present in the Guadiana estuary. In spite of the co-occurrence of anchovy spawning (Ré, 1996) and the *B. virginica* medusa seasonal cycle in the Mira estuary (present study), the predation impact on anchovy eggs probably is not significant, as the frequency of this prey item in *B. virginica* guts was very low; however the fixed sampling site established in the present study was not at the center of the anchovy egg distribution in the estuary, which usually is present closer to the river mouth (Ré, 1987, 1996).

5. Conclusions

The seasonal cycle of the hydromedusa *Blackfordia virginica* was confirmed in the Mira estuary. The medusae occurred in the warmer periods of the year and they were first observed after the zooplankton outbreak in the estuary. As documented in other systems, the medusae consumed zooplankton, ingesting the most abundant organisms available in the water column, copepods. The medusae potential predation impact on the copepod population in the Mira estuary may be significant, as low half-life of copepods were obtained. However, a high vacuity index may suggest that *B. virginica* medusae may have a complementary diet, which could include smaller organisms or suspended organic matter, as noted by other authors (Morais et al., 2015). Thus, further investigation is needed to better understand the feeding habits and the predatory mechanisms of *B. virginica* medusae, as well as the dynamics of the main prey organisms, in order to estimate more accurately the medusae predation impact in invaded systems.

Acknowledgments

This study was supported by the projects UID/MAR/04292/2013 and 3M RECITAL (LTER/BIA-BEC/0019/2009) funded by FCT. Paula Chainho was also funded by an FCT post-doctoral grant (SFRH/BDP/29579/2006). We thank Mr. Rafael, Mr. Vitalino and his sons for the assistance during the sampling in the Mira estuary, Elisabete Henriques, Lurdes Dias, Pedro Freitas, Ana Matias and Nora for helping in the laboratory work and Patrícia Presado and Marta Correia for helping with figures.

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