

Research Article

Food sources of the non-indigenous bivalve *Ruditapes philippinarum* (Adams and Reeve, 1850) and trophic niche overlap with native species

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Abstract

The Manila clam, *Ruditapes philippinarum* (Adams and Reeve, 1850), was introduced in many estuaries along the Atlantic and Mediterranean coasts for fisheries and aquaculture, being one of the top five most commercially valuable bivalve species worldwide. In Portugal, the colonization of the Tagus estuary by this species coincided with a significant decrease in abundance of the native *R. decussatus* (Linnaeus, 1758). This study aimed at identifying the main food sources supporting populations of the non-native bivalve in the Tagus estuary, using carbon and nitrogen stable isotopes, and evaluate the potential for food competition with the native bivalves *R. decussatus* and *Cerastoderma glaucum* (Bruguière, 1789). Results showed that these species relied on the same food sources, and that the trophic niche of *R. philippinarum* overlapped with the trophic niche of *R. decussatus* by 40% and with *C. glaucum* by 23%. The most likely food sources included particulate organic matter (POM), microphytobenthos (MPB), and sediment organic matter (SOM). The Bayesian stable isotope mixing model indicated that POM was the food source with the highest proportional contribution (up to 92%), followed by MPB (up to 32%), and SOM (up to 23%). Although the majority of the food sources identified were filtered from the water column, reliance on SOM and MPB suggests they may also feed on resuspended organic matter. Because these bivalve species feed on the same sources, there is some potential for food competition in this ecosystem. However, further studies are needed to analyze the long-term consequences of these trophic interactions to verify if the co-existence between the native and the invasive species will generate competition for food resources when those are limited in quantity and/or quality.

Key words: Manila clam, non-indigenous species, *Ruditapes decussatus*, *Cerastoderma glaucum*, stable isotopes, Tagus estuary

Introduction

Estuaries are among the most altered ecosystems by non-indigenous species (NIS; Williams and Grosholz 2008). Once NIS are established in a new environment, they can become invasive by changing local biological community interactions, causing reductions in the abundance of native

populations or even extinctions, and modifying ecosystem functioning (Ruiz et al. 1997). Impacts can be caused directly by biotic interactions with the resident community (e.g., competition, predation, introduction of pathogens) and indirectly by changes in habitat conditions (e.g., turbidity, habitat structure) (Crooks 2002).

Bivalves are one of the most invasive groups, as many species can rapidly establish at remarkably high densities, sometimes accounting for the majority of local benthic faunal biomass (Sousa et al. 2009). The Manila clam, *Ruditapes philippinarum* (Adams and Reeve, 1850), is distributed worldwide (Suh and Shin 2013), and is now one of the top five most commercially valuable bivalve species across the world (Chiesa et al. 2016). It is native to the Indo-Pacific region, but it has been introduced in many estuaries along the Atlantic and Mediterranean coasts for fisheries and aquaculture (Chiesa et al. 2016). It was introduced between 1972 and 1975 in France and later in England, Spain, and Italy (Flassch and Leborgne 1992; Humphreys 2010). In Portugal, its presence has been known for more than two decades and it has been observed in several estuaries (e.g., the Tagus and Sado River) and coastal lagoons (e.g., Ria Formosa and Ria de Aveiro) (Campos and Cachola 2006).

This species has a high salinity tolerance (14–34; Carregosa et al. 2014), and it is able to colonize both brackish and marine habitats. High densities have been documented in several European estuaries: 497 ind.m⁻² in the Venice lagoon, Italy (Brusà et al. 2013), up to 592 ind. m⁻² in the Tagus estuary, Portugal (Garaulet 2011), and higher than 1000 ind. m⁻² in the Adriatic Sea, Italy (Breber 2002). The invasion success of *R. philippinarum* has been attributed to life history characteristics such as fast growth rates, an extended breeding season, and a high number of offspring (Moura et al. 2017, 2018). Moreover, this species has a high filtering capacity (0.96 dm³.h⁻¹.ind⁻¹; Sorokin and Giovanardi 1995), feeding on plankton and other microorganisms (Nakamura 2001). This species can also feed on benthic organic matter sources (Suh and Shin 2013; Zhao et al. 2013), potentially favouring the competition for food with native filter feeding bivalve species.

Due to their feeding plasticity and high densities in invaded ecosystems, these clams have the potential to alter trophic relationships and influence the fluxes of organic matter (OM) in aquatic food webs. For example, biodeposition by invasive bivalves can directly affect benthic-pelagic coupling and the downward flux of OM (e.g., Crooks and Khim 1999; Gergs et al. 2009; Norkko and Shumway 2011).

Carbon ($\delta^{13}\text{C}$: $^{13}\text{C}/^{12}\text{C}$) and nitrogen ($\delta^{15}\text{N}$: $^{15}\text{N}/^{14}\text{N}$) stable isotope analysis is a powerful tool to characterize energy flow through aquatic food webs (Layman et al. 2012). This approach is based on the relationship between the isotopic composition of dietary resources in the ecosystem and the isotopic composition of consumers' tissues that incorporate these dietary resources into their structural components and energy reserves

(Peterson and Fry 1987). Thus, the stable isotope ratio of a consumer reflects its diet, demonstrating an average trophic fractionation (i.e., the difference between the consumer and its diet) of *ca.* +0.5‰ $\delta^{13}\text{C}$ and +3.4‰ $\delta^{15}\text{N}$ per trophic level (Vander Zanden and Rasmussen 2001), although with some variability around these values (Caut et al. 2009).

The present study aimed at determining the food sources supporting the production of *R. philippinarum* and of the native bivalve species, *R. decussatus* (Linnaeus, 1758) and *Cerastoderma glaucum* (Bruguière, 1789), in the Tagus estuary (central western coast of Portugal), and investigating the potential trophic niche overlap between species. *Ruditapes philippinarum* is the dominant bivalve species in some areas of the Tagus estuary, particularly in shallow bays with extensive intertidal areas (Ramajal et al. 2016). The colonization of the Tagus estuary by this species coincided with a sharp decrease in the abundance of the native *R. decussatus* (Chainho 2014). The reasons for this decrease may include competition for the same resources and habitat, and/or the decrease in *R. decussatus* abundance due to long term economic exploitation, which opened a window for the establishment of the NIS *R. philippinarum*.

Because *R. philippinarum*, *R. decussatus*, and *C. glaucum* occupy the same ecological niches in the Tagus estuary (Garaulet 2011) we hypothesized they will also feed on the same food sources. To test this hypothesis, we identified their potential food sources and quantified their relative contribution to the bivalve species' biomass in the Seixal Bay (Tagus estuary) using C and N stable isotope analysis. Knowing the food sources utilized by these non-native and native species can advance our understanding on the potential for resource competition.

Materials and methods

Study area

The Tagus estuary is located in the central western-Iberian Peninsula (southwest-Europe; Figure 1) and it is one of the largest estuaries on the west coast of Europe covering *ca.* 320 km² (Cotter et al. 2013). It is a semi-diurnal mesotidal estuary, with tidal range varying from 1 m during neap tides up to almost 4 m in the spring tides. The tide propagates up to 80 km upstream and the mean water residence time in the estuary is 19 days (Braunschweig et al. 2003; Dias et al. 2013). The Tagus estuary can be divided into three main areas: a straight narrow and deep west-east oriented channel about 16 km long and 2 km wide, with maximum depths of about 45 m; an inner bay 25 km long and 15 km wide, oriented southwest-northeast, with depths between 5 and 10 m; and an upper shallow estuary with an area of 100 km² with large mudflats and salt marshes separated by shallow channels (Braunschweig et al. 2003). The Seixal bay is a peripheral embayment, located in the southern part of the Tagus

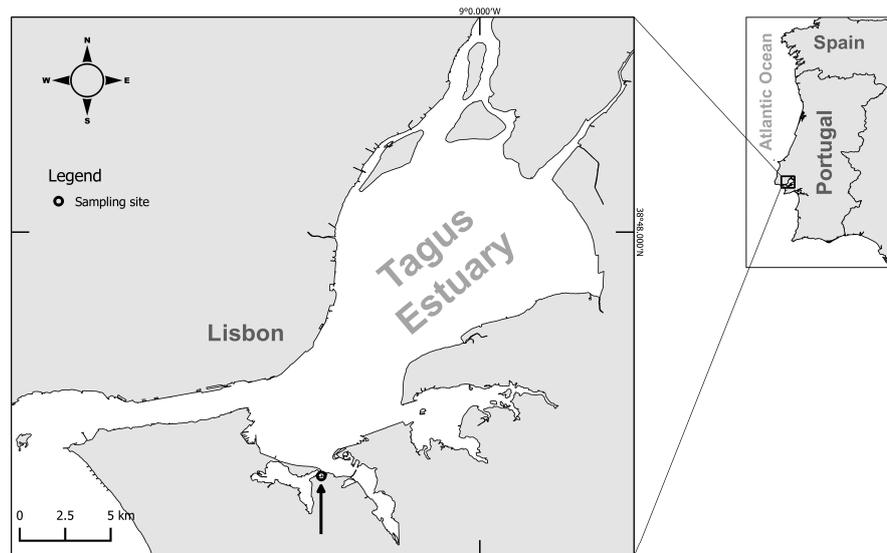


Figure 1. Location of the sampling station in the Seixal Bay (Tagus estuary).

estuary (Figure 1) where salinity usually varies between 20 and 25 (Garaulet 2011). This area shows nutrients enrichment (Caetano et al. 2016) and is highly contaminated by metals and metalloids (Chiesa et al. 2018) due to local inputs associated with harbour, urban, and industrial activities (Mil-Homens et al. 2014).

Field sampling

Samples were collected in the Seixal Bay during low tide of spring tides (Figure 1) in October 2015, to characterize low river discharge conditions (SNIRH 2018). During low river discharge, salt water intrusion is maximized, favoring connectivity between the estuary and the marine environment, and residence time is prolonged, which allows phytoplankton biomass to accumulate (Sin et al. 1999). This sampling area was selected based on the fact it presents high density values of *R. philippinarum* and where *R. decussatus* density values were also high before the invasion of *R. philippinarum* (Carvalho 2016).

Ten individuals from each species, *R. philippinarum*, *R. decussatus*, and *C. glaucum*, were hand collected from the sediment, with gloves, and kept frozen at $-20\text{ }^{\circ}\text{C}$ until analysis. From each species, only adult individuals were sampled ($> 20\text{ mm}$ *C. glaucum*, Derbali et al. 2014; $> 30\text{ mm}$ *R. decussatus* and *R. philippinarum*, Derbali et al. 2016; Moura et al. 2018; Table 1) in order to avoid potential isotopic variability due to ontogeny (Minagawa and Wada 1984).

The potential food sources for the bivalves were selected based on their feeding modes which include filter feeding on particles in the water column and on particles resuspended from the sediment (Kasai et al. 2004; Watanabe et al. 2009; Suh and Shin 2013): particulate organic matter (POM), sediment organic matter (SOM), microphytobenthos (MPB), macroalgae, and saltmarsh vegetation. At each station only surface water samples were

collected because sampled locations were shallow (less than 0.5 m). From these samples, the concentration of Chl *a* and concentration and isotopic composition of POM were measured (mg L^{-1} , $\delta^{13}\text{C}_{\text{POC}}$, $\delta^{15}\text{N}_{\text{PN}}$, molar C:N). Replicate POM and Chl *a* water samples (POM: 1 L, Chl *a*: 0.5 L) were pre-filtered with a 38 μm , 63 μm , and 150 μm sieves and filtered onto pre-combusted (500 °C for 2 h) Whatman GF/F and Whatman GF/C filters, respectively, and kept frozen (−20 °C) until analysis. Microphytobenthos samples were obtained following Eaton and Moss' (1966) method of active migration to light through lens tissue (in Vafeiadou et al. 2013), in which the upper 3 mm of the surface was scratched (biofilms must show brown or green colours). A small portion of the biofilm was preserved in formalin for later microscopic analysis and the remaining biofilm was stored frozen for stable isotope analyses (Vafeiadou et al. 2013). Sediment, and the most abundant macroalgae (*Enteromorpha* sp. and *Fucus vesiculosus*), and plants (*Juncus maritimus*, *Spartina maritima*, and *Halimione portulacoides*) were hand collected using gloves. Plants and macroalgae were washed with ultrapure water to remove epiphytes, and dried at 60 °C for at least 48 h.

Laboratory analysis

Filters collected for Chl *a* analysis were extracted in 90% acetone and analyzed on a Spectronic 20 Genesys spectrophotometer. Chl *a* concentration was calculated following Lorenzen(1967).

Filters for POM and MPB analysis were cut in half for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analyses. Before being dried at 60 °C for 24 h, the half for $\delta^{13}\text{C}$ analysis was fumigated with concentrated HCl for 12 hours, to remove inorganic carbonates (Lorrain et al. 2003). Sediment subsamples were rinsed with 10% HCl, to remove carbonates, and dried at 60 °C for 48 h (Lorrain et al. 2003).

The shell length of each bivalve specimen was measured (± 0.01 mm). Then, the foot was excised, dried at 60 °C, and ground to a fine powder with a mortar and pestle for stable isotope analysis. Muscle tissues have slower turnover rates and less lipids than other tissues such as gonads and digestive glands (Paulet et al. 2006; Malet et al. 2007) and thus, are considered better indicators of long-term diet (Cabanellas-Reboredo et al. 2009). Macroalgae, plants, and sediment samples were also ground to a fine powder with a mixer mill.

Stable isotope ratios were measured using a Thermo Scientific Delta V Advantage IRMS via Conflo IV interface (Marinnova, University of Porto). The raw data were normalized by three-point calibration using international reference materials, such as IAEA-N-1 ($\delta^{15}\text{N} = +0.4\text{‰}$), IAEA-NO-3 ($\delta^{15}\text{N} = +4.7\text{‰}$), and IAEA-N-2 ($\delta^{15}\text{N} = +20.3\text{‰}$) for nitrogen isotopic composition, and two-point calibration using USGS-40 ($\delta^{13}\text{C} = -26.39\text{‰}$) and USGS-24 ($\delta^{13}\text{C} = -16.05\text{‰}$) for carbon isotopic composition. Stable isotope ratios are reported in δ notation, $\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3$, where X is the C or N stable isotope, R is the ratio of heavy/light stable isotopes. The $\delta^{13}\text{C}$

and $\delta^{15}\text{N}$ are expressed in units per mill (‰) relative to Vienna Pee Dee Belemnite and air, respectively. The analytical error, the mean SD of replicate reference material, was $\pm 0.1\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Data analysis

To test for possible differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between species a one-way PERMANOVA was used. In the PERMANOVA test, the statistical significance of variance ($\alpha = 0.05$) was tested using 9999 permutations of residuals within a reduced model. When the number of permutations was lower than 150, the Monte Carlo p value was considered. Statistical tests were conducted using the PRIMER software (v.6.1.6, PRIMER-E) with the permutational multivariate analysis of variance (PERMANOVA) + 1.0.1 add-on (Anderson et al. 2008).

The contribution of the different food sources to bivalve tissues were estimated using the Bayesian isotope mixing model SIAR (Stable Isotope Analysis in R), a package in R (R Core Team 2018). The model allows each of the sources and the trophic enrichment factor (TEF; or trophic fractionation) to be assigned as a normal distribution (Parnell et al. 2010). SIAR will produce a range of feasible solutions to the mixing problem to which are assigned credibility intervals (CIs) (in this study, 95% CI; Parnell et al. 2010). For the mixing model, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were adjusted for one trophic level using the trophic fractionation estimates from Vander Zanden and Rasmussen (2001: $+0.47 \pm 1.2\text{‰}$ $\delta^{13}\text{C}$, $+3.4 \pm 0.2\text{‰}$ $\delta^{15}\text{N}$), which are similar to those obtained in feeding experiments with juveniles of *R. philippinarum* reared on microalgae ($+0.6$ $\delta^{13}\text{C}$, $+3.4\text{‰}$ $\delta^{15}\text{N}$; Yokoyama et al. 2005).

To estimate the niche width for each species the bivalves' stable isotope data were used to calculate the standard ellipse area (SEA) using the SIBER package (Jackson et al. 2011) which is also part of the open source statistical language R (R Core Team 2018). SEA is a bivariate measure of the distribution of individuals in the trophic space; each ellipse encloses *ca.* 95% of the data (Jackson et al. 2011, 2012). To account for variation in sample sizes, we calculated a Bayesian estimate of SEA (SEA_B) using Markov chain Monte Carlo simulation with 10^4 iterations for each group (Jackson et al. 2011). When overlap was observed between the sympatric bivalves' ellipses, then the extent of overlap (%) was also calculated to indicate the extent of resource sharing.

Bivalve $\delta^{13}\text{C}$ values were corrected for lipid content because lipids are depleted in ^{13}C when compared to protein and carbohydrates which usually results in an inverse relationship between C:N and $\delta^{13}\text{C}$ in muscle tissues for aquatic animals (DeNiro and Epstein 1977). Variability in lipid content can influence bulk tissue $\delta^{13}\text{C}$ values and thereby cause dietary or habitat shifts to be incorrectly interpreted (Logan et al. 2008). The average (\pm SD) bivalve C:N values were higher than 4 (Table 1) indicating some lipid content (Post et al. 2007; Hoffman and Sutton 2010). Bivalve muscle tissue

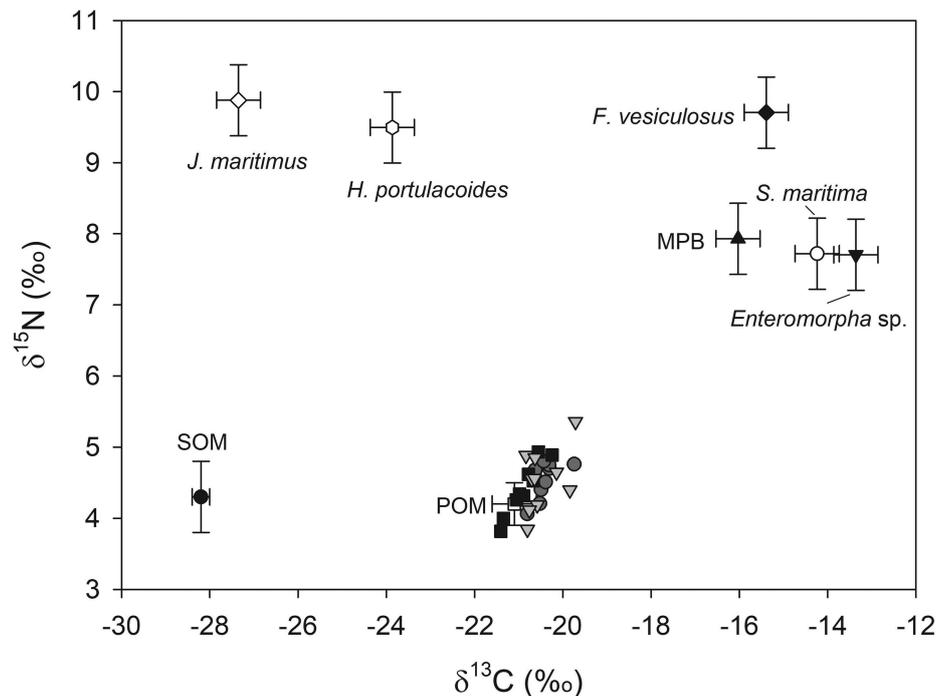


Figure 2. Average (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *Ruditapes philippinarum* (closed squares), *Ruditapes decussatus* (dark grey circle), and *Cerastoderma glaucum* (light grey inverted triangle) adjusted for one trophic level fractionation ($+0.47\text{‰}$ for $\delta^{13}\text{C}$, $+3.4\text{‰}$ for $\delta^{15}\text{N}$) and potential organic matter sources. Error bars represent one standard deviation. The sources considered relevant for bivalves were microphytobenthos (MPB), sediment organic matter (SOM), particulate organic matter (POM), marsh plants (*Juncus maritimus*, *Spartina maritima*, and *Halimione portulacoides*), and macroalgae (*Enteromorpha* sp. and *Fucus vesiculosus*).

data were corrected for lipid content using tissue C:N following the mass balance correction for fish muscle tissue proposed by Hoffman and Sutton (2010; their Eq. 6), which uses estimates of $\text{C:N}_{\text{protein}}$ and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ that are similar to those from the muscle tissue found for other fish (e.g., Sweeting et al. 2006) and taxonomic groups (e.g., shrimp and zooplankton; Fry et al. 2003; Smyntek et al. 2007).

Results

The average (\pm SD) chlorophyll *a* (Chl *a*) concentration was $4.7 \pm 2.7 \mu\text{g L}^{-1}$ while the average (\pm SD) particulate organic carbon (POC) concentration was $0.33 \pm 0.04 \text{ mg L}^{-1}$. The average (\pm SD) of particulate organic matter (POM) C:N (molar) was 6.5 ± 1.1 .

The food sources were in general isotopically well differentiated (Figure 2). Among sources, the macroalgae *F. vesiculosus* and *Enteromorpha* sp. presented the highest average (\pm SD) $\delta^{15}\text{N}$ ($9.7 \pm 0.5\text{‰}$) and $\delta^{13}\text{C}$ values ($-13.4 \pm 0.5\text{‰}$), respectively (Figure 2). The source with the lowest average (\pm SD) $\delta^{15}\text{N}$ value was POM ($4.2 \pm 0.3\text{‰}$), whereas SOM was the source with the lowest average (\pm SD) $\delta^{13}\text{C}$ value ($-28.2 \pm 2\text{‰}$) (Figure 2). The $\delta^{13}\text{C}_{\text{POC}}$ values from the POM fractions $< 38 \mu\text{m}$, $38\text{--}63 \mu\text{m}$, $63\text{--}150 \mu\text{m}$, and $< 150 \mu\text{m}$ ranged between -21.4‰ ($< 150 \mu\text{m}$) and -20.4‰ ($< 38 \mu\text{m}$) and the $\delta^{15}\text{N}_{\text{PN}}$ values varied between 3.8‰ ($38\text{--}63 \mu\text{m}$) and 4.5‰ ($< 38 \mu\text{m}$).

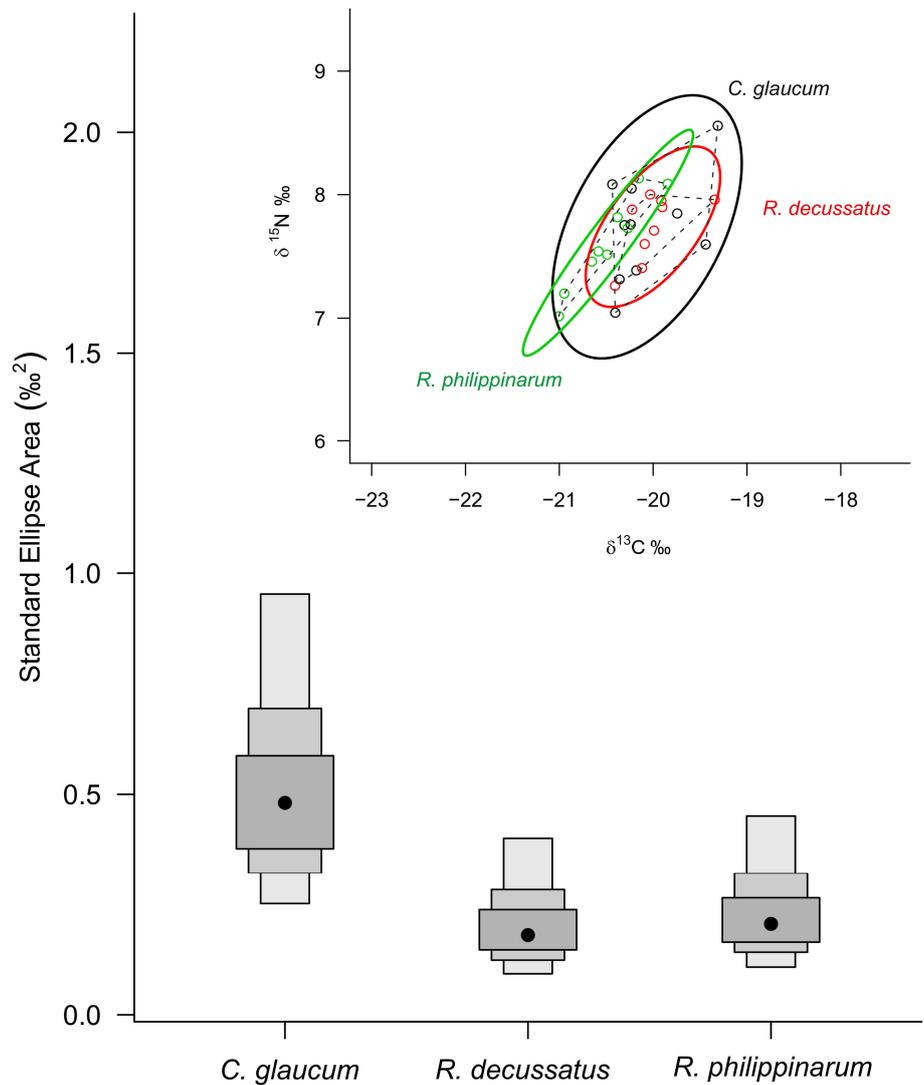


Figure 3. Standard ellipse area ($\%^{2}$) of *Cerastoderma glaucum*, *Ruditapes decussatus*, and *Ruditapes philippinarum*, based on their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Black dots indicate the mode values and boxes indicate 50%, 75%, and 95% Bayesian credible intervals. Top right: stable isotopes bi-plot illustrating the trophic niche width of each bivalve species, represented by the solid line ellipses.

Table 1. Average size (mm), $\delta^{13}\text{C}$ ($\%$), $\delta^{15}\text{N}$ ($\%$), and C:N (\pm SD) values of the bivalve species collected in the Seixal Bay in October 2015.

Consumer	Size (mm)	$\delta^{13}\text{C}$ ($\%$)	$\delta^{15}\text{N}$ ($\%$)	C:N
<i>Ruditapes philippinarum</i>	40.7 (3.2)	-20.5 (0.4)	7.6 (0.4)	4.4 (0.1)
<i>Ruditapes decussatus</i>	45.7 (5.2)	-20.0 (0.3)	7.7 (0.3)	4.7 (0.2)
<i>Cerastoderma glaucum</i>	27.7 (1.2)	-20.1 (0.4)	7.7 (0.4)	4.8 (0.6)

Because the differences in the stable isotope ratios between fractions were small (up to 1‰), we used the average (\pm SD) values of the fraction $< 150\ \mu\text{m}$ ($\delta^{15}\text{N}_{\text{PN}}$: $4.2 \pm 0.3\%$; $\delta^{13}\text{C}$: $-21.4 \pm 0.5\%$) in the stable isotope mixing model.

The stable isotopes values of the bivalves collected (Table 1) were not statistically different (Pseudo-F: 2.65; $P > 0.05$) and their ranges overlapped (Figure 2), which suggests that these species were feeding on the same food sources. The trophic niche size (95% CI) of *C. glaucum* was the largest, followed by *R. philippinarum* and *R. decussatus* (Figure 3). The trophic niche

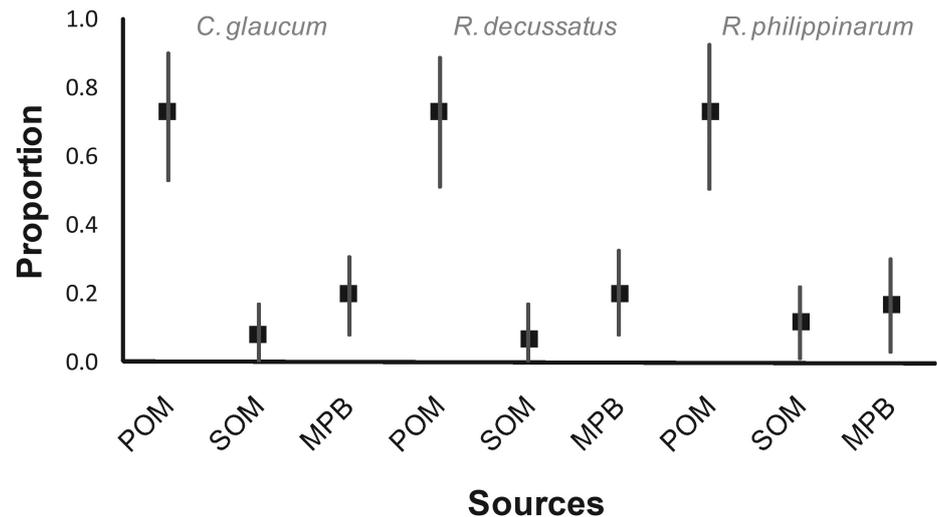


Figure 4. Proportion of each food source to the bivalves' biomass—*Cerastoderma glaucum*, *Ruditapes decussatus*, and *Ruditapes philippinarum*—in the Seixal Bay (Tagus estuary) based on the stable isotope mixing model. The food sources included in the model were particulate organic matter (POM), sediment organic matter (SOM), and microphytobenthos (MPB). Closed squares indicate the most likely value (mode) and lines indicate the 95% Bayesian credibility intervals.

of *R. philippinarum* overlapped with the trophic niche of *C. glaucum* (23%) and *R. decussatus* (40%). The trophic niche of *R. decussatus* overlapped in 40% with the trophic niche of *C. glaucum*. Based on the biplot analysis, the most important food source contributing to the bivalves' biomass in the Seixal Bay was the POM, because their stable isotope values fall inside the range of $\delta^{15}\text{N}_{\text{PN}}$ and $\delta^{13}\text{C}_{\text{POC}}$ values, after correcting for trophic fractionation (Figure 2). However, some individuals presented $\delta^{15}\text{N}$ values slightly higher than it would be expected if they were only filter feeding on POM. This indicates they assimilated a more ^{15}N -enriched source, which include plants and macroalgae detritus and/or MPB (Figure 2). We consider MPB to be the most plausible source because phytoplankton is a more labile source (Deegan and Garritt 1997) and because previous studies indicate that *R. philippinarum* feed on benthic phytoplankton (Suh and Shin 2013 and references there in). Based on the isotopic mixing model (95% CI) the source contributing the most to the bivalves' biomass was the POM varying between 53% and 90% for *C. glaucum*, 52% and 89% for *R. decussatus*, and between 50% and 92% for *R. philippinarum* (Figure 3). The contribution of MPB varied between 7% and 31% to the *C. glaucum*'s biomass, 8% and 32% to the *R. decussatus*'s biomass, and between 3% and 29% to the *R. philippinarum*'s biomass (Figure 4). The contribution of SOM was low and up to 23% (Figure 4).

Discussion

The spread of NIS has been identified as a major ecological threat in coastal marine communities (Claudet and Fraschetti 2010). Non-indigenous bivalve species can rapidly attain high density values (Carlton et al. 1990; Sousa et

al. 2009) with potential to cause several ecological and economic impacts (Sousa et al. 2009, 2011, 2014; Crespo et al. 2015). Nowadays, *R. philippinarum* is the dominant bivalve species in some areas of the Tagus estuary, particularly in shallow bays with extensive intertidal area (as is the area in this study) (Moura et al. 2018).

This study indicates that in the Seixal Bay, the main food source contributing to the bivalves' biomass was the particulate organic matter (POM) with proportional contributions up to 90%. This POM pool was likely comprised mostly of phytoplankton due to fact that: 1) the C/N_{POM} ratio was 6.5, which is close to the Redfield ratio for phytoplankton (6.6); 2) the $\delta^{13}\text{C}_{\text{POC}}$ values ($-21.1 \pm 0.5\text{‰}$) were similar to the $\delta^{13}\text{C}$ values for marine phytoplankton ($-20.5 \pm 1.3\text{‰}$, McMahon et al. 2013); and 3) Chl *a* average concentrations were *ca.* two times higher than those registered in this estuary during autumn (average 1999–2005: $2.4 \pm 1.7 \mu\text{g L}^{-1}$; Gameiro et al. 2007), but similar to summer concentrations (average 1999–2010: $4.0 \mu\text{g L}^{-1}$; Brito et al. 2015). Moreover, sampling was conducted at the end of the typical low river inflow period (SNIRH 2018), when residence time is elongated, and phytoplankton biomass accumulates (Hoffman and Bronk 2006).

Though POM was the main food source for these bivalve species, microphytobenthos (MBP) and sediment organic matter (SOM) also supplemented their diet with relative contributions up to 32% and 23%, respectively. The most likely mechanism by which they accessed benthic organic matter was by filtering resuspended particles in the water column, because they were collected in shallow subtidal areas (Watanabe et al. 2009). Benthic organic matter was described as the most important food source for *R. philippinarum* in several ecosystems in Japan (Watanabe et al. 2009) and Korea (Kang et al. 2007; Suh and Shin 2013). The fact they can access both to organic matter in the water column and in the sediments, indicates they are generalist species. Although we did not measure the primary productivity in the Tagus estuary during the year of sampling, the MPB productivity is estimated to be more than the double than the phytoplankton productivity (Brotas and Catarino 1995; Cabeçadas 1999), and thus a higher proportional contribution of MPB to the bivalves' biomass would be expected.

The trophic niche of the non-native bivalve *R. philippinarum* and the native bivalves *C. glaucum* and *R. decussatus* overlapped because they assimilated the same food sources and in similar proportions. Thus, all species can obtain food both from the water column and from the sediments. Although the potential for food competition is high in this ecosystem, this should be analyzed cautiously. A fundamental question concerning the feeding interactions between invasive and native species is whether invasion will increase interspecific competition, with negative consequences for the competing species, or if trophic niche divergence occurs, which would facilitate the invader's integration into the community

and their coexistence with the native species. The niche variation hypothesis predicts that under interspecific competition, populations become less generalized in their diet (Van Valen 1965), which often results in the decrease of the niche width from native populations (Human and Gordon 1996; Thomson 2004; Olsson et al. 2009), but it is also expected that competing invasive species will occupy smaller niches than their allopatric counterparts (Jackson et al. 2012). On the other hand, it was hypothesized that increased competition for resources can result in larger trophic niches enabling species to maintain their energy requirements (Svanbäck and Bolnick 2007). With this study we cannot conclude on the long-term consequences of the feeding interactions between the invasive and the native bivalve species in the Tagus estuary, but our preliminary analysis shows that the species with the lowest trophic niche width was the invasive species and that diet overlap was observed between the three species. These findings differ from studies where trophic niche divergence was observed between invasive and functionally similar native fish species both in the field and in controlled conditions (Tran et al. 2015). One possible explanation may be related with an apparent abundance of high quality food sources in this ecosystem during low river discharge conditions, including phytoplankton in the water column and MPB (Brotas and Catarino 1995; Cabeçadas 1999). Thus, a similar study should be conducted during winter conditions, when the quality of the estuarine POM pool is expected to be low due to an increase in the proportional contribution of terrestrial-derived organic matter (Hoffman et al. 2008; Dias et al. 2016). Also, the number of habitats surveyed should increase in order to cover all the areas where these species co-occur which would provide a better understanding if feeding strategies vary according with the type of habitat and type of food available. Previous studies have shown that *R. philippinarum* can assimilate terrestrial-derived organic matter, although its proportional contribution tends to be low (Kasai et al. 2004). Thus, this species has the ability to use food sources from different origins. This flexibility can enhance its potential for competition with native bivalve species. Moreover, to the best of our knowledge, there are no studies indicating if the native *R. decussatus* and *C. glaucum* can also assimilate low quality food sources such as terrestrial-derived organic matter in natural environments.

Many native bivalve species have been displaced and reduced in their distribution areas (Pranovi et al. 2006; Castorani and Hovel 2015). Although the mechanisms leading to the increase in the abundances and dispersion of *R. philippinarum* in the Tagus estuary are not clear, it was observed that it coincided with a sharp decrease in the abundance of *R. decussatus* (Chainho et al. 2015). This might have occurred due to interspecific competition for resources (Chainho et al. 2015) or due to the fact that *R. decussatus* was intensively harvested in the past leaving the habitats vacant to be colonized by *R. philippinarum* (Garaulet 2011), or both. Changes in ecological,

chemical or physical features of the ecosystem can facilitate the invasion process by non-indigenous species (NIS; Cloern 2001). For instance, the sharp increase in the abundance and biomass of the invasive freshwater bivalve *Corbicula fluminea* in the Minho river estuary (NW-Portugal) was followed by an event of high mortality due to a heat wave, from which the sympatric native Unionidae species did not recover (Ronaldo Sousa, *personal communication*). The extreme weather event likely created a mismatch between the traits in native species and their environment, so that *C. fluminea*, due to its life history characteristics including rapid individual growth, high fecundity, and broad environmental tolerance (Morton 1997), was better positioned to occupy the habitats left by the native species (Byers 2002). A similar phenomenon might have occurred in the Venice lagoon with the invasion of *R. philippinarum* (Pranovi et al. 2006). It was observed that before the demographic explosion of this NIS, the ecosystem was heavily stressed due to anoxia and high ammonia concentrations in the benthic compartment, which inhibited benthic invertebrate populations (Sfriso et al. 2003; Pranovi et al. 2006). Hybridization between native and invasive species can also be a threat to the native species populations (Sousa et al. 2014). Although some genetic studies conducted in the *Ruditapes* populations in Spain have raised the possibility of hybridization between *R. decussatus* and *R. philippinarum* (Hurtado et al. 2011), molecular studies conducted in the Portuguese populations did not show any evidence of hybridization, including in the Tagus estuary (Chiesa et al. 2017).

A critical assumption in the SIAR mixing model is that the food source values measured were similar over the prior 2–4 months because the data collected during this study were obtained during a single sampling event. Although isotopic turnover rate studies of the analyzed species are not available, it is likely that the isotopic turnover period of these species is within that timeframe. The isotopic turnover period depends on both somatic growth and metabolic turnover rates; in organisms that grow rapidly, somatic growth rates essentially determine the isotopic turnover period (del Rio et al. 2009). *Cerastoderma glaucum*, *R. decussatus*, and *R. philippinarum* have rapid growth throughout their life (Kandeel et al. 2017; Moura et al. 2017). For example, the cockle *Cerastoderma edule* is expected to turnover within weeks (whole-body half-life of *ca.* 7 days for $\delta^{13}\text{C}$ and 8 days for $\delta^{15}\text{N}$; Lefebvre and Dubois 2017). Moreover, previous studies have demonstrated that stable isotope values of other bivalves *Dreissena polymorpha* (zebra mussel) and *Corbula amurensis* (Asian clam; formerly known as *Potamocorbula*) are spatially and temporally well aligned with environmental processes (e.g., watershed inputs, chemistry of river water; Fry 2002; Fry and Allen 2003). The same is likely true for the analyzed species owing to its rapid growth rate and similarly short lifespan

(McMahon 2002; Thompson and Parchaso 2010; Silina 2011; Bensaâd-Bendjedid et al. 2018). The C and N stable isotope values of estuarine basal organic matter sources usually change faster than those from its consumers, seasonally over annual cycles of production and senescence, and with physical mixing and river discharge magnitude (Cloern et al. 2002; Hoffman and Bronk 2006). Sampling occurred at the beginning of autumn, in the lower portion of the estuary, during low flow conditions that usually persist over the summer months (Gameiro et al. 2007; SNIRH 2018), a timeframe similar to the expected turnover period of these species. Also, during low flow conditions, the riverine and terrestrial inputs are usually low, when compared to high flow conditions, and relatively constant (Hoffman and Bronk 2006; Hoffman et al. 2008). Thus, it is likely that the biogeochemical conditions in October were similar to the summer low flow biogeochemical conditions that preceded sampling.

Another assumption in the SIAR mixing model is that the trophic fractionation values of these species are similar to those estimated by Vander Zanden and Rasmussen (2001: $+0.47 \pm 1.2\text{‰}$ $\delta^{13}\text{C}$, $+3.4 \pm 0.2\text{‰}$ $\delta^{15}\text{N}$). Although, to the best of our knowledge, there are no estimates for the native species analyzed in this study, Yokoyama et al. (2005) estimated trophic fractionation values for juvenile *R. philippinarum* reared on microalgae ($+0.6$ $\delta^{13}\text{C}$, $+3.4\text{‰}$ $\delta^{15}\text{N}$), which are similar to those used in this study. Because the ontogenetic stage (Minagawa and Wada 1984) and type of diet (Caut et al. 2009) can influence the trophic fractionation values, we used the estimates from Vander Zanden and Rasmussen (2001), which are also similar to those obtained for other bivalves species such as the freshwater clam *Corbicula sandai* (Kasai et al. 2016).

In conclusion, this study revealed that there is trophic niche overlap between the native bivalve species *C. glaucum* and *R. decussatus* and the invasive bivalve *R. philippinarum* in the Tagus estuary, because they assimilated the same sources. Although POM was the source with the highest contribution to their biomass, this study showed they can access to benthic OM (MPB and SOM), thus sharing similar feeding strategies. Nonetheless, further studies are needed to analyze the long-term consequences of these feeding interactions to verify if the co-existence between the native and the invasive species tends to generate competition for food resources, especially when they are low in quantity and/or quality.

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