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RESEARCH ARTICLE

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Trophic niche overlap between native freshwater mussels (Order: Unionida) and the invasive *Corbicula fluminea*

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Abstract

1. Freshwater mussels (Order Unionida) are highly threatened. Interspecific competition for food sources with invasive alien species is considered to be one of the factors responsible for their decline because successful invaders are expected to have wider trophic niches and more flexible feeding strategies than their native counterparts.
2. In this study, carbon ($\delta^{13}C$: $^{13}C/^{12}C$) and nitrogen ($\delta^{15}N$: $^{15}N/^{14}N$) stable isotopes were used to investigate the trophic niche overlap between the native freshwater mussel species, *Anodonta anatina*, *Potomida littoralis*, and *Unio delphinus*, and the invasive bivalve *Corbicula fluminea* living in sympatry in the Tua basin (south-west Europe).
3. The species presenting the widest trophic niches were *C. fluminea* and *A. anatina*, which indicate that they have broader diets than *U. delphinus* and *P. littoralis*. Nonetheless, all the species assimilated microphytobenthos, sediment organic matter, and detritus derived from vascular plants, although with interspecific variability in the assimilated proportions of each source. The trophic niche of the invasive species overlapped with the trophic niche of all the native species, with the extent varying between sites and according to the species.
4. From the three native species analysed, *Potomida littoralis* may be at a higher risk for competition for food with *C. fluminea* in the Tua basin, if food sources become limited, because this native mussel presented the narrowest trophic niche across sites and the highest probability of overlapping with the trophic niche of *C. fluminea*.

Ester Dias has equal contribution as first author.

5. Given the global widespread distribution of *C. fluminea*, the implementation of management measures devoted to the control or even eradication of this invasive alien species should be a conservation priority given its potential for competition with highly threatened native freshwater mussels.

KEYWORDS

Asian clam, invasive alien species, stable isotopes, sympatry, trophic interactions

1 | INTRODUCTION

The introduction of invasive alien species (IAS) is one of the main global threats to biodiversity and ecosystem functioning (Simberloff & Rejmánek, 2011; Gutiérrez, Jones & Sousa, 2014; IPBES, 2019). Trophic interactions such as predation and competition for food with native species are among the mechanisms influencing the successful colonization of a new habitat by IAS (Thomson, 2004; Baiser, Russell & Lockwood, 2010; Jackson et al., 2012). Also, IAS with flexible feeding strategies and generalized diets might have greater chances of successfully colonizing a new habitat than specialist species, because food availability is less likely to be a limiting factor (Moyle & Light, 1996). However, a fundamental question regarding the feeding interactions between native and invasive species occupying similar trophic niches is whether an invasion will increase the interspecific competition for food, with adverse consequences for native species (Crowl, Townsend & McIntosh, 1992), or whether trophic niche divergence occurs, which could facilitate their coexistence (Tran et al., 2015). The niche variation hypothesis predicts that under interspecific competition, populations become less generalized in their diet (Van Valen, 1965); thus, the niches of native species may become narrower after an invasion (Thomson, 2004). By contrast, Svanbäck & Bolnick (2007) hypothesized that increased competition for food sources can result in wider trophic niches, enabling species to maintain their energy requirements. These contrasting ecological outcomes are usually difficult to investigate owing to the lack of information about the food web structure before the introduction of an IAS. Nonetheless, the analysis of trophic niche sizes and overlap between native and invasive species can provide some indication of the potential for competition when food sources are limited (Olsson et al., 2009; Tran et al., 2015; Larson, Twardochleb & Olden, 2017).

Originating from Southeast Asia, the Asian clam *Corbicula fluminea* (Müller, 1774) is a ubiquitous and extremely versatile invader in freshwater ecosystems (Crespo et al., 2015). This species is considered opportunistic because of its short life cycle, rapid growth, early sexual maturity, high fecundity, and phenotypic plasticity (Sousa, Antunes & Guilhermino, 2008; Sousa et al., 2014). In contrast, populations of the previously dominant freshwater mussels in European ecosystems belonging to the order Unionida have severely declined in recent decades primarily as a result of habitat loss and degradation, over-exploitation, climate change, and the introduction of IAS (Lopes-Lima et al., 2017; Lopes-Lima et al., 2018; Modesto et al., 2018), which in some cases may be acting synergistically. Understanding the impacts of each threat can be critical to mitigate their effects and to apply more effective management measures for freshwater mussel conservation (Ferreira-Rodríguez et al., 2019). In fact, the introduction of *C. fluminea* has been identified as a factor with sufficient magnitude to impair the conservation of several freshwater mussel species (Strayer, 1999; Vaughn & Hakenkamp, 2001; Modesto et al., 2019).

A mechanism by which this IAS might affect native freshwater mussels unfavourably is through competition for food because they have similar feeding strategies and feed on similar food sources (Novais, Dias & Sousa, 2016). They filter-feed on bacteria, phytoplankton, and detrital material available in the water column, and also on particles deposited in the sediments (McMahon, 1991; Raikow & Hamilton, 2001; Vaughn & Hakenkamp, 2001; Vaughn, Spooner & Galbraith, 2007; Vaughn, Nichols & Spooner, 2008; Dias et al., 2016). Filter-feeding is generally accepted as the main mechanism used by *C. fluminea* and freshwater mussels to obtain food (Hakenkamp & Palmer, 1999; Vaughn & Hakenkamp, 2001; Vaughn, Nichols & Spooner, 2008; Dias et al., 2014). However, pedal-feeding (a type of deposit-feeding) has been described for *C. fluminea* and unionid juveniles as another strategy to obtain food from the sediments (Hakenkamp & Palmer, 1999; Nichols & Garling, 2000; Nichols et al., 2005).

Several studies have already addressed the feeding ecology of *C. fluminea* (Boltovskoy, Izaguirre & Correa, 1995; Nichols et al., 2005; Dias et al., 2014; Bolam, Rollwagen-Bollens & Bollens, 2019) and freshwater mussels (Raikow & Hamilton, 2001; Nichols et al., 2005; Mistry & Ackerman, 2018), but the trophic niche space occupied by these species when living in sympatry and the potential for competition for food sources has rarely been investigated (but see Novais, Dias & Sousa, 2016). This study aimed to investigate the trophic niche occupied by the native freshwater mussels *Anodonta anatina* (Linnaeus, 1758), *Potomida littoralis* (Cuvier, 1798), and *Unio delphinus* (Spengler, 1793) that live in sympatry with the invasive *C. fluminea* in the Tua River basin (south-west Europe). The specific aims were (i) to estimate species' trophic niche width; (ii) to determine the trophic niche overlap between species; and (iii) to identify and quantify the food sources assimilated by each species. To accomplish these objectives, carbon ($\delta^{13}C$: $^{13}C/^{12}C$) and nitrogen ($\delta^{15}N$: $^{15}N/^{14}N$) stable isotopes were used because they provide a time-integrated signal of the food sources in the ecosystem that is incorporated into the structural components and energy reserves of consumers (Peterson & Fry, 1987). Thus, stable isotopes can be used not only to identify the sources assimilated by each species, but also to provide information on niche width, representing the diversity of food sources assimilated within populations or species (Jackson et al., 2011). Because the native freshwater mussels and *C. fluminea* occupy similar ecological niches in the Tua basin (Sousa et al., 2012) and also because they can feed on similar food sources and have similar feeding strategies (McMahon, 1991; Vaughn & Hakenkamp, 2001; Vaughn, Spooner & Galbraith, 2007; Vaughn, Nichols & Spooner, 2008; Dias et al., 2014; Novais, Dias & Sousa, 2016), we hypothesized that the trophic niche overlap would be high between the invasive and the native species.

2 | METHODS

2.1 | Study area

The Rabaçal and Tuela Rivers are tributaries of the Tua River located in the north-west Iberian Peninsula (south-west Europe; Figure 1). The Tua River has a total length of 142 km and its basin an area of 3,813 km^2 (690 km^2 in Spain and 3,123 km^2 in Portugal) (Sousa et al., 2012). The Rabaçal River has a length of 80 km and the Tuela River has a length of 102 km (Sousa et al., 2018). These three rivers are well-preserved, and have high conservation value owing to the low level of human pressures (Sousa et al., 2012; Sousa et al., 2015; Sousa et al., 2019), which probably contributes to the presence of several endemic and threatened species including plants (e.g. *Festuca duriotagana*, Franco & Rocha Afonso, 1981), freshwater mussels (e.g. *Margaritifera margaritifera*, Linnaeus, 1758; *P. littoralis* and *U. delphinus*), fishes (e.g. *Cobitis calderoni*, Bacescu, 1962; *Pseudochondrostoma duriense*, Coelho, 1985; *Squalius alburnoides*, Steindachner, 1866), and mammals (e.g. *Microtus cabrerae*, Thomas, 1906; *Galemys pyrenaicus*, Geoffroy, 1811) (ICNF, 2020).

2.2 | Field collection

Sampling was conducted in October 2016 and May 2017, before and after the rainy season, respectively. Native freshwater mussels (*A. anatina*, *P. littoralis*, and *U. delphinus*), the invasive *C. fluminea*, and their potential food sources were collected at five sites in the Tua basin: sites 1 and 2 in the Tua River, sites 3 and 4 in the Rabaçal River, and site 5 in the Tuela River (Figure 1). These sites encompass the distribution range of the invasive species living in sympatry with the native bivalves in the Tua basin (Sousa et al., 2012; Sousa et al., 2020). *Corbicula fluminea* was present at all sites; *A. anatina* at sites 1, 2, and 4; *P. littoralis* at sites 1, 2, and 3; and *U. delphinus* at sites 1, 2, 4, and 5.

Ten individuals of each species were hand-collected at each sampling site, at depths of 1–2 m in areas of 50 m^2 and up to 2 m

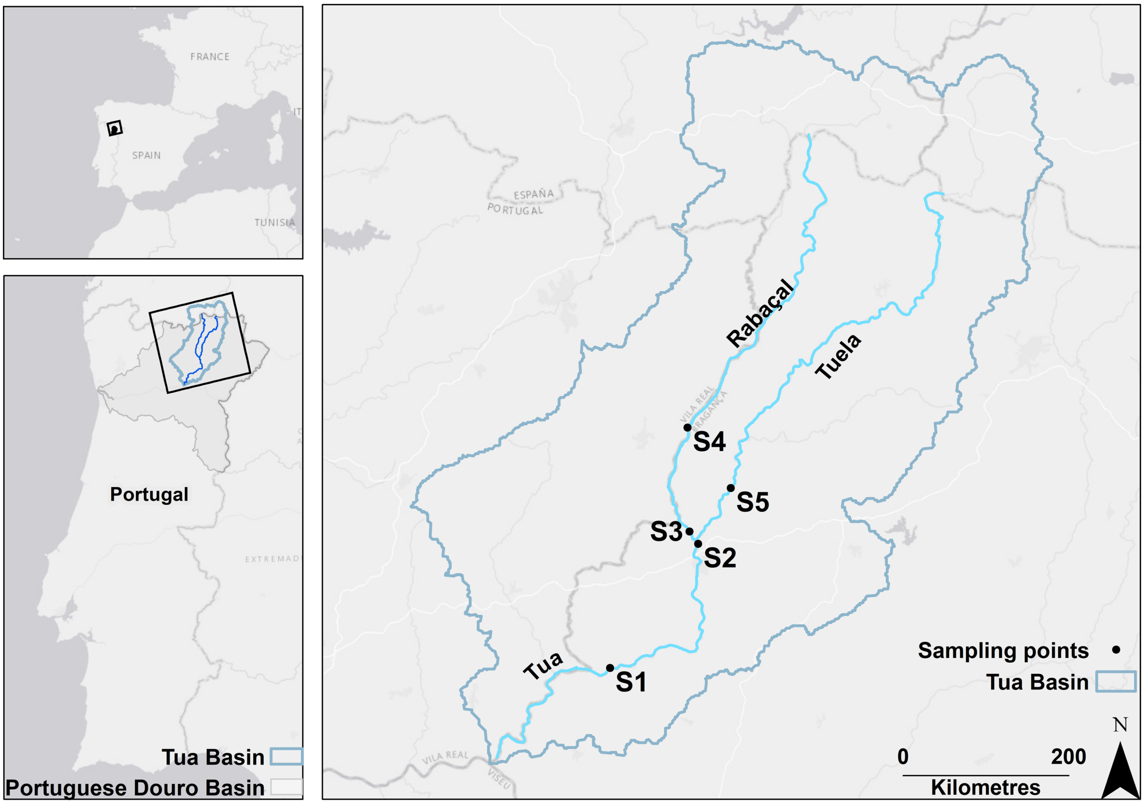

FIGURE 1 Location of the sampling sites in the Tua basin (north-west Iberian Peninsula)

from the river banks. An exception occurred for *A. anatina* at site 4. Given its reduced density at this site, only three and five individuals were collected before and after the rainy season, respectively. The shell length (mm) of each individual was recorded and a small portion of the foot was excised for stable isotope analysis. Afterwards, the individuals belonging to the native species were carefully returned to their original habitats. Foot-muscle tissue samples were frozen (−20°C) for subsequent analyses.

Potential food sources for bivalves were selected based on their feeding modes (i.e. filter-feeding and pedal-feeding; Hakenkamp & Palmer, 1999; Vaughn, Nichols & Spooner, 2008; Atkinson et al., 2011; Dias et al., 2014) and included: particulate organic matter (POM), microphytobenthos (MPB), sediment organic matter (SOM), terrestrial-derived organic matter (Terr), and emergent (EAV) and submerged (SAV) aquatic vegetation.

To determine the isotopic composition of POM ($\delta^{13}C_{POC}$, $\delta^{15}N_{PN}$) replicate (n = 2) surface water samples were collected at each site (0.5 m above the substrate) and stored on ice until processing at the laboratory. Water samples were pre-filtered through a 150-μm sieve and filtered onto pre-combusted (500°C for 2 h) Whatman GF/F filters. Samples were frozen (−20°C) until analysis. MPB was collected from artificial substrates (PVC pipe — 5 cm diameter; 50 cm length), which were fixed in the sediment and left for 2 weeks before sampling. At the end of this period, colonizing algae were scraped from the substrate into a clean vial with deionized water and stored on ice until processing at the laboratory where the same procedure used for POM samples was applied.

Sediments and the most abundant plants (terrestrial and aquatic) were hand-collected using gloves. Sediments were collected 2 m from the river banks, by excavating to a depth of ca. 5 cm. Terrestrial plants such as *Agrostis stolonifera* (Linnaeus), *Alnus glutinosa* (Linnaeus) Gaertn., *Arundo donax* (Linnaeus), *Osmunda regalis* (Linnaeus), *Polygonum lapathifolium* (Linnaeus), *Pteridium aquilinum* (Linnaeus) Kuhn, and *Salix salviifolia* (Brot.) were collected in the river banks. Aquatic plants (emergent and submerged) were also collected when present. Emergent aquatic plants included *Carex elata* subsp. *reuteriana* (Boiss.) Luceño & Aedo, *Cyperus longus* (Linnaeus), and *Leersia oryzoides* (Linnaeus) Swartz, while *Ranunculus peltatus* (Schrank) was the only submerged plant present at the sampling sites. Samples were stored on ice and returned to the laboratory where they were kept frozen (−20°C) for subsequent processing.

2.3 | Laboratory analyses

Filters for POM and MPB analysis were cut in half and one half was fumed with concentrated HCl (to remove inorganic carbonates) and dried (60°C for 24 h) to determine $\delta^{13}C_{POC}$ (following Lorrain et al., 2003). The other half was only dried (60°C for 24 h) to determine $\delta^{15}N_{PN}$. Sediment samples (SOM) were ground to a fine powder, and the sub-sample used to determine $\delta^{13}C_{SOC}$ was rinsed with 10% HCl (to remove carbonates) and dried (60°C for 48 h). The other sub-sample was only dried (60°C for 48 h) to determine the $\delta^{15}N_{SN}$. Plants were washed with deionized water and dried (60°C for 48 h). Sediments and plants were ground to a fine powder with a mixer mill for stable isotope analysis. The foot-muscle tissue of each bivalve was dried (60°C) and ground to a fine powder with a mortar and pestle for stable isotope analyses.

Stable isotope ratios were measured using a Thermo Scientific Delta V Advantage IRMS via a 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 ($\delta^{15}N$ = +0.4‰), IAEA-NO-3 ($\delta^{15}N$ = +4.7‰), and IAEA-N-2 ($\delta^{15}N$ = +20.3‰) for nitrogen isotopic composition, and two-point calibration using USGS-40 ($\delta^{13}C$ = −26.39‰) and USGS-24 ($\delta^{13}C$ = −16.05‰) for carbon isotopic composition. Stable isotope ratios are expressed in δ notation, $\delta X = (R_{sample}/R_{standard} - 1) \times 10^3$, where X is the C or N stable isotope, and R is the ratio of heavy: light stable isotopes. The $\delta^{13}C$ and $\delta^{15}N$ values are expressed in units per mill (‰) relative to Vienna Pee Dee Belemnite and air, respectively. Based on the mean standard deviation (SD) of replicate reference material, the observed analytical error of $\delta^{13}C$ and $\delta^{15}N$ was ±0.15‰. To control for sample processing quality, samples with SD between replicates (i.e. two sub-samples of the same sample) >0.2‰ of $\delta^{13}C$ and $\delta^{15}N$ were not included in subsequent analyses. The $\delta^{13}C$ of bivalve species were corrected for lipid content using the mass balance correction proposed by Hoffman and Sutton (2010, Eq. 6) because lipids are ^{13}C-depleted when compared with proteins and carbohydrates, which can influence bulk tissue $\delta^{13}C$ values (DeNiro & Epstein, 1977; Logan et al., 2008).

2.4 | Data analyses

To test for differences in the shell length of each bivalve species between different sites and seasons, a two-way ANOVA was used, with sites (five levels: [S1], [S2], [S3], [S4], and [S5]) and seasons (two levels: [before] and [after] the rainy season) as fixed factors. The assumptions of normality and homogeneity of variance were tested. The statistical significance of variance was α = 0.05. Shell lengths were modelled with the *aov* function from the package MASS (Venables & Ripley, 2002). Tukey multiple pairwise-comparisons were calculated with the *glht* function in the package multcomp (Bretz, Hothorn & Westfall, 2010).

To determine the possible differences in the $\delta^{13}C$ and $\delta^{15}N$ values of each bivalve species between different sites and seasons, generalized linear models (GLMs) were fitted to the data using the Gaussian distribution (following Zuur et al., 2009). Each model included a response variable, $\delta^{13}C$ or $\delta^{15}N$ values, and two categorical explanatory variables: sites (five levels: [S1], [S2], [S3], [S4], and [S5]) and seasons (two levels: [before] and [after] the rainy season). In addition, because the species' shell lengths varied between and within sites, this variable was included as a continuous variable in the models. The residuals of the model were checked considering the assumptions of normal and homogeneous error. The statistical significance of variance used in the models was α = 0.05. The $\delta^{13}C$

and δ^{15}N values were modelled with the *glm* function in the package MASS (Venables & Ripley, 2002). Pairwise comparisons of least-square means were calculated with the *lsmeans* function in the package lsmeans (Lenth, 2016).

To estimate the trophic niche width for each bivalve species, the stable isotope values were used to calculate the standard ellipse area (SEA) using the SIBER (Stable Isotope Bayesian Ellipses in R) package (Jackson et al., 2011). The SEA is a bivariate measure of the distribution of individuals in the trophic space (Jackson et al., 2011). To account for the small sample size, the corrected standard ellipse areas (SEA_C; considering 40% of central data points) and the corresponding 95% Bayesian ellipse areas (SEA_B) were estimated for each species per site (Jackson et al., 2011; Jackson et al., 2012). When overlap was observed between the trophic niche of the invasive species (*C. fluminea*) and the native bivalves (*A. anatina*, *P. littoralis*, and *U. delphinus*), the extent of overlap (%) was calculated using the SEAc, which represents the overlap between the core dietary niches of any pair of species (Jackson et al., 2011; Jackson et al., 2012). In addition, the probability of pairwise overlap (%) was calculated using the nicheROVER package (Swanson et al., 2015), which calculates the probability of the niche of one species overlapping with the niche of another species, using 95% credible intervals and a Monte Carlo estimation with 10^4 steps for each pair (Swanson et al., 2015). Thus, the pairwise overlap was calculated considering the probability of an individual of *C. fluminea* to be found in the trophic niche of each native species.

To estimate the contribution of the different food sources to the bivalves' tissues the Bayesian dual-stable isotope mixing model SIAR (Stable Isotope Analysis in R) was used to solve the indeterminate equations (where the number of sources is greater than the number of isotopes plus 1), producing a probability distribution that represents the likelihood a given food source contributes to the consumer's biomass (Parnell et al., 2010). The model allows each of the sources and the trophic enrichment factor (or trophic fractionation) to be assigned as a normal distribution, rather than a single datum (Parnell et al., 2010) and produces several viable solutions to which are assigned credibility intervals (in this study, 95% CI; Parnell et al., 2010). For the mixing model, the δ^{13}C and δ^{15}N values were adjusted for one trophic level using the trophic fractionation estimates from Vander Zanden and Rasmussen (2001: +0.47 ± 1.2‰ δ^{13}C, +3.4 ± 0.2‰ δ^{15}N).

For modelling purposes (SIBER, nicheROVER, and SIAR), the information collected in each season was integrated and analyses were only performed by site. *Corbicula fluminea* is expected to turn over more quickly (weeks to a few months), owing to rapid growth throughout their life (McMahon, 2002; Kasai, Ishizaki & Isoda, 2016), than Unionida, which have longer life cycles with slower growth rates (Hinzmann et al., 2013; Reátegui-Zirena et al., 2013; Lopes-Lima et al., 2017; Lopes-Lima et al., 2020). To the best of our knowledge, there are no estimates of isotopic turnover rates available for the species analysed, but the turnover of the unionid *Pleurobema sintoxia* has been estimated to be approximately 1 year (357 days; Raikow & Hamilton, 2001). Thus, we expected the stable isotope ratios from the freshwater mussels analysed during this study to result from the integration of the seasonal isotopic variability in their potential food sources. Individuals (*C. fluminea* at site 1 and *A. anatina* at site 2) that apparently were not in equilibrium with the food sources available (after adjusting for trophic fractionation), i.e. showing δ^{13}C and δ^{15}N values outside the isotopic space formed by the δ^{13}C and δ^{15}N values of the food sources collected, were not included in the SIAR mixing models.

All the analyses were performed using the open-source statistical language R software (R Development Core Team, 2014).

3 | RESULTS

3.1 | Biotic characterization

Shell length varied between species, with *C. fluminea* and *U. delphinus* showing smaller sizes than *A. anatina* and *P. littoralis* (Figure 2a,b). Shell length of each species varied between sites but not between seasons (Figure 2a,b). The ANOVA models showed significant differences between sites for *C. fluminea* (F = 13.888, df = 4; $P < 0.05$), *A. anatina* (F = 4.230, df = 2; $P < 0.05$), *P. littoralis* (F = 31.090, df = 2; $P < 0.05$), and *U. delphinus* (F = 31.993, df = 3; $P < 0.05$).

The δ^{13}C values varied between species and sites. *Corbicula fluminea* and *P. littoralis* presented higher δ^{13}C values than *A. anatina* and *U. delphinus* (Figure 2c,d). Except for *A. anatina*, all species showed significant differences in their δ^{13}C values between sites (Table 1). *Corbicula fluminea* and *P. littoralis* were more ^{13}C-depleted at sites 4 and 2, respectively, whereas *U. delphinus* displayed the highest δ^{13}C values at site 5 (Figure 2c; Table 1). Only *U. delphinus* showed significant differences in the δ^{13}C values between seasons, being more ^{13}C-enriched after the rainy season (Figure 2d; Table 1). The δ^{13}C values did not vary with shell length (Table 1). The δ^{15}N values also varied between species and sites. *Unio delphinus* exhibited higher δ^{15}N values than the other species and, except for *A. anatina*, the δ^{15}N values of each species varied between sites (Figure 2e; Table 2). There were no significant variations in the δ^{15}N values of the bivalves between seasons (Figure 2f; Table 2). The δ^{15}N values did not vary with shell length, except for *P. littoralis* (Table 2).

3.2 | Isotopic niche width and overlap

In general, *C. fluminea* and *A. anatina* displayed wider trophic niches than the other species (Table 3) with *C. fluminea* having wider trophic niches than all the native species living in sympatry at sites 1, 3, and 5 (Table 3). At sites 2 and 4, *C. fluminea* exhibited smaller trophic niches than *A. anatina* (Table 3). *Potomida littoralis* and *U. delphinus* presented the smallest trophic niches at all sites (Table 3). The extent of overlap between the core trophic niche of *C. fluminea* and the native species varied between species and sites (Table 3). The overlap was the highest with *A. anatina* at site 1 (99%), but decreased

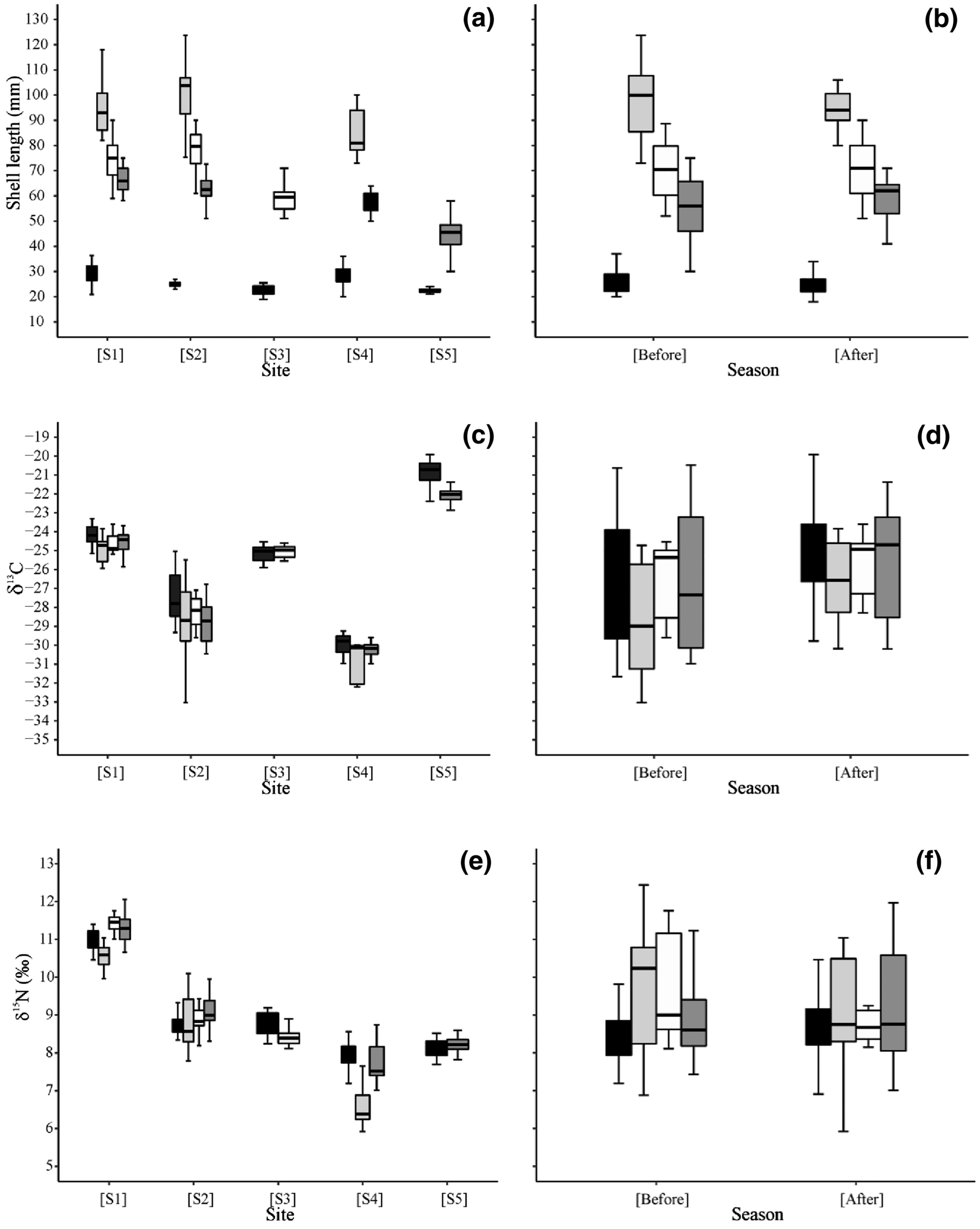

FIGURE 2 Boxplots of the (a,b) shell length (mm), (c,d): $\delta^{13}C$ (‰), and (e,f): $\delta^{15}N$ (‰) of *Corbicula fluminea* (black), *Anodonta anatina* (light grey), *Potomida littoralis* (white), and *Unio delphinus* (dark grey) collected at five sites (S1, S2, S3, S4, and S5) in the Tua basin before (October 2016) and after the rainy season (May 2017). Error bars represent one standard deviation

upstream (16% at site 2, and 0% at site 4; Table 3). Similarly, the overlap between the core trophic niche of *U. delphinus* and *C. fluminea* decreased upstream (35% at site 1, 12% at site 2, and 0% at site 5), except at site 4, where the overlap was 76% (Table 3). The overlap between the core trophic niche of *P. littoralis* and *C. fluminea* followed the opposite trend, with an overlap of 9% at site 1, and 59% and 61% at sites 2 and 3, respectively (Table 3). However, the probability of *C. fluminea* to overlap with the trophic niche of *P. littoralis* was always high, varying between 88% at site 1 and 94% at site 3 (Table 3). In contrast, the probability of *C. fluminea* to overlap with the trophic niche of *A. anatina* was high only at site 1 (99%), decreasing upstream (33% at site 2, and 7% at site 4; Table 3). A similar pattern was observed for *U. delphinus*, with the probability of *C. fluminea* overlapping with the trophic niche of this species varying between 92% at site 1 and 58% at site 5 (Table 3). The exception was for site 4, where the probability of *C. fluminea* to overlap with the trophic niche of *U. delphinus* was 88% (Table 3).

3.3 | Food sources

The stable isotope values for bivalves, after adjusting for trophic fractionation, were generally intermediate between the food sources analysed, indicating reliance on multiple food sources (Figure 3). Exceptions were observed for some individuals belonging to *C. fluminea* (two individuals) and *A. anatina* (four individuals) at sites 1 and 2, respectively, which were ^{13}C-depleted or ^{15}N-enriched, or both, when compared with the sources sampled (Figure 3).

TABLE 1 Generalized linear model outputs of the model fitted to quantify the variation of δ^{13}C ratios in response to the shell length of the freshwater bivalve individuals collected (*Corbicula fluminea*, *Anodonta anatina*, *Potomida littoralis*, and *Unio delphinus*) in the different sites (S1, S2, S3, S4, and S5) of the Tua basin before (October 2016) and after the rainy season (May 2017)

	Estimate	Std. error	*t* value	Pr (>|*t*|)
Corbicula fluminea				
(Intercept)	−23.56	1.72	−13.71	*<0.050*
ShellLength	−0.07	0.06	−1.17	0.246
[S2]	−4.65	2.45	−1.90	0.061
[S3]	−0.91	3.28	−0.28	0.783
[S4]	−7.22	2.22	−3.25	*<0.050*
[S5]	5.25	3.42	1.54	0.128
Season[After]	0.11	1.36	0.08	0.936
Anodonta anatina				
(Intercept)	−26.00	1.53	−17.00	<0.050
ShellLength	0.00	0.02	−0.01	0.995
[S2]	−0.29	2.66	−0.11	0.913
[S4]	−5.17	3.34	−1.55	0.130
Season[After]	0.04	2.19	0.02	0.985
Potomida littoralis				
(Intercept)	−24.58	0.97	−25.34	*<0.050*
ShellLength	−0.01	0.01	−0.39	0.700
[S2]	−3.40	1.26	−2.70	*<0.050*
[S3]	−0.34	1.07	−0.32	0.754
Season[After]	−0.16	0.67	−0.25	0.807
Unio delphinus				
(Intercept)	−26.06	0.82	−31.85	*<0.050*
ShellLength	0.01	0.01	1.05	0.300
[S2]	−3.55	1.35	−2.64	*<0.050*
[S4]	−2.26	1.65	−1.37	0.176
[S5]	6.05	1.01	5.97	*<0.050*
Season[After]	−1.53	0.72	−2.11	*<0.050*

Note: The use of italics indicates the significance of the test, that is, <0.050.

TABLE 2 Generalized linear model outputs of the model fitted to quantify the variation of δ^{15}N ratios in response to the shell length of the freshwater bivalve individuals collected (*Corbicula fluminea*, *Anodonta anatina*, *Potomida littoralis*, and *Unio delphinus*) in the different sites (S1, S2, S3, S4, and S5) of the Tua basin before (October 2016) and after the rainy season (May 2017)

	Estimate	Std. error	*t* value	Pr (>|*t*|)
Corbicula fluminea				
(Intercept)	11.08	0.89	12.40	*<0.050*
ShellLength	−0.02	0.03	−0.69	0.495
[S2]	−3.86	1.27	−3.04	*<0.050*
[S3]	−3.24	1.71	−1.90	0.061
[S4]	−3.89	1.16	−3.37	*<0.050*
[S5]	−3.86	1.78	−2.17	*<0.050*
Season[After]	−1.01	0.71	−1.43	0.155
Anodonta anatina				
(Intercept)	9.56	1.35	7.08	*<0.050*
ShellLength	0.01	0.01	0.93	0.358
[S2]	−0.38	2.35	−0.16	0.871
[S4]	−4.47	2.95	−1.52	0.138
Season[After]	−0.87	1.93	−0.45	0.655
Potomida littoralis				
(Intercept)	10.09	0.66	15.31	*<0.050*
ShellLength	0.02	0.01	2.07	*<0.050*
[S2]	−1.52	0.86	−1.77	0.082
[S3]	−1.51	0.73	−2.07	*<0.050*
Season[After]	−0.16	0.45	−0.37	0.712
Unio delphinus				
(Intercept)	10.00	0.74	13.60	*<0.005*
ShellLength	0.02	0.01	1.74	0.085
[S2]	−1.06	1.21	−0.88	0.385
[S4]	−2.72	1.48	−1.83	0.071
[S5]	−2.20	0.91	−2.41	*<0.050*
Season[After]	−0.88	0.65	−1.35	0.183

Note: The use of italics indicates the significance of the test, that is, <0.050.

Based on the biplot analysis, stable isotope ratios for bivalves showed a reliance on ^{13}C- and ^{15}N-enriched sources such as MPB, and POM at sites 1, 3, and 5, whereas at sites 2 and 4, bivalves seemed to rely more on ^{13}C-depleted and ^{15}N-depleted sources such as detritus derived from vascular plants (Figure 3). At site 2, the variability in the δ^{13}C values of all bivalves indicated a reliance on multiple sources, from ^{13}C-enriched sources such as MPB to more ^{13}C-depleted sources such as detritus from terrestrial plants (Figure 3).

The isotopic mixing models (95% CIs) indicate that the proportional contribution of each food source to bivalve biomass varied between sites (Table 4). At site 1, biomass was mostly supported by MPB (up to 90% for *P. littoralis*; Table 4). Detritus from EAV was also an important source for *A. anatina* (19%–46%) and *C. fluminea* (11%–31%; Table 4). At site 2, the main contributors to *C. fluminea* biomass were SOM (26%–51%) and detritus from Terr (17%–42%; Table 4). Detritus from Terr was also the source with the highest contribution to the biomass of *P. littoralis* (41%–76%) and *U. delphinus* (52%–79%; Table 4). At site 3, SOM was the source contributing the most to bivalve biomass (79%–93% for *P. littoralis* and 80%–99% for *C. fluminea*; Table 4). At site 4, the sources with the highest contribution to bivalve biomass were detritus from Terr (up to 83% for *A. anatina*) and EAV (up to 72% for *C. fluminea*; Table 4). At site 5, the main contributors to the biomass of *C. fluminea* were SAV (36%–59%) and MPB (27%–51%) and for *U. delphinus* were SAV (34%–54%) and POM (24%–42%; Table 4).

TABLE 3 Corrected (SEA$_C$; ‰) and Bayesian standard ellipse area (SEA$_B$; ‰^{2}) of *Corbicula fluminea*, *Anodonta anatina*, *Potomida littoralis*, and *Unio delphinus*, collected at five different sites (S1, S2, S3, S4, and S5) in the Tua basin (south-west Europe)

Site	Species	SEA$_C$ (‰)	SEA$_B$ (‰^{2})	SEA$_C$ overlap (%)	Pairwise overlap (%)
[S1]	*Corbicula fluminea*	3.13	6.61 (4.05–10.30)	-	-
	Anodonta anatina	0.67	0.30 (0.20–0.51)	99	97
	Potomida littoralis	0.35	0.31 (0.18–0.53)	9	88
	Unio delphinus	0.57	0.52 (0.32–0.85)	35	92
[S2]	*Corbicula fluminea*	1.55	1.31 (0.68–1.74)	-	-
	Anodonta anatina	7.26	8.40 (5.35–14.05)	16	33
	Potomida littoralis	0.85	0.76 (0.48–1.20)	61	93
	Unio delphinus	1.45	1.29 (0.83–2.08)	12	68
[S3]	*Corbicula fluminea*	0.71	0.64 (0.39–1.04)	-	-
	Potomida littoralis	0.24	0.22 (0.14–0.35)	59	94
[S4]	*Corbicula fluminea*	0.96	0.77 (0.49–1.23)	-	-
	Anodonta anatina	1.19	0.82 (0.42–1.93)	0	7
	Unio delphinus	0.50	0.48 (0.29–0.79)	76	88
[S5]	*Corbicula fluminea*	0.57	0.53 (0.32–0.84)	-	-
	Unio delphinus	0.39	0.35 (0.22–0.56)	0	58

Note: Estimated trophic niche overlap between the core trophic niche of each native species (*A. anatina*, *P. littoralis*, and *U. delphinus*) and the invasive *C. fluminea* (SEA$_C$ overlap; %) and the probability of an individual of the invasive *C. fluminea* to be found in the trophic niche of each native species (pairwise overlap; %).

4 | DISCUSSION

The invasive *C. fluminea* and the native freshwater mussel *A. anatina* displayed wider trophic niches than the other species. The trophic niche of the invasive species overlapped with the trophic niche of all native mussels in the Tua basin, although the extent of the overlap varied between sites and species. *Potomida littoralis* showed the narrowest trophic niche and the highest probability to overlap with the trophic niche of *C. fluminea*.

The trophic niche width of *C. fluminea* and native freshwater mussels varied according to the site. Specifically, *C. fluminea* showed a wider trophic niche than *P. littoralis* at all sites, and similar to (sites 2 and 5) or wider than (sites 1 and 4) *U. delphinus*. The wider trophic niche of *C. fluminea* is in agreement with several other studies showing that invasive species have, in general, wider trophic niches than their native counterparts, suggesting higher trophic flexibility and broader diets (Schmid, 1984; Morton, 1997; Olsson et al., 2009; Guzzo et al., 2013). A similar result was observed also in the Ichawaynochaway Creek (USA), where the trophic niche of *C. fluminea* was found to be wider than that of the sympatric native freshwater mussel *Elliptio crassidens* (Atkinson et al., 2010). Thus, a broader diet may constitute an advantage for the successful invasion of *C. fluminea* when competing for food sources with co-occurring native species. The fact that *C. fluminea* presented overall wider trophic niches than the native species may explain the high probability values (>60%) of overlap with the trophic niches of the native species. However, contrary to this general pattern, *A. anatina* exhibited a wider trophic niche than *C. fluminea* at sites 2 and 4. At these sites, the probability of *C. fluminea* to overlap with the trophic niche of *A. anatina* varied between 33% and 7%, respectively, which suggests some trophic divergence. In fact, the overlap between their core diet was low, varying between 0% and 16%, which indicates that their main diet was different or that they were feeding on different proportions of the same sources. *Potomida littoralis*, by contrast, was the species with the narrowest trophic niche among the species analysed, at all sites, suggesting a less flexible diet. It was also the species with the overall highest probability values of trophic niche overlap with *C. fluminea*, except at site 1. Although the pairwise overlap estimate was 88%, the overlap between the core trophic niche of these species was only 9%. This means that although their core diet is less similar because *C. fluminea* has a wider trophic niche, the probability of an individual of *C. fluminea* to be found in the trophic niche of *P. littoralis* is high. Similar results were found for *U. delphinus*, especially at sites 2 and 5.

In a previous study, where interspecific and intraspecific variability in the δ^{15}N and δ^{13}C values from *C. fluminea* and native freshwater mussels were evaluated across a range of ecosystems in Portugal subjected to different environmental conditions (e.g. phytoplankton availability and organic pollution), no trophic niche overlap was observed between *C. fluminea* and the native freshwater mussels in the Tua River (site 1 in this study, Novais, Dias & Sousa, 2016), contrary to the results obtained in the present study. Moreover, trophic niche overlap seemed to occur especially in ecosystems with low phytoplankton availability (Paiva and Minho rivers, Novais, Dias & Sousa, 2016). One possible explanation for the differences may be related to the fact that the study by Novais, Dias & Sousa (2016) was based on a single sampling event, during the summer of 2012 when food availability could have been different. According to optimal foraging theories, trophic niche increases as a

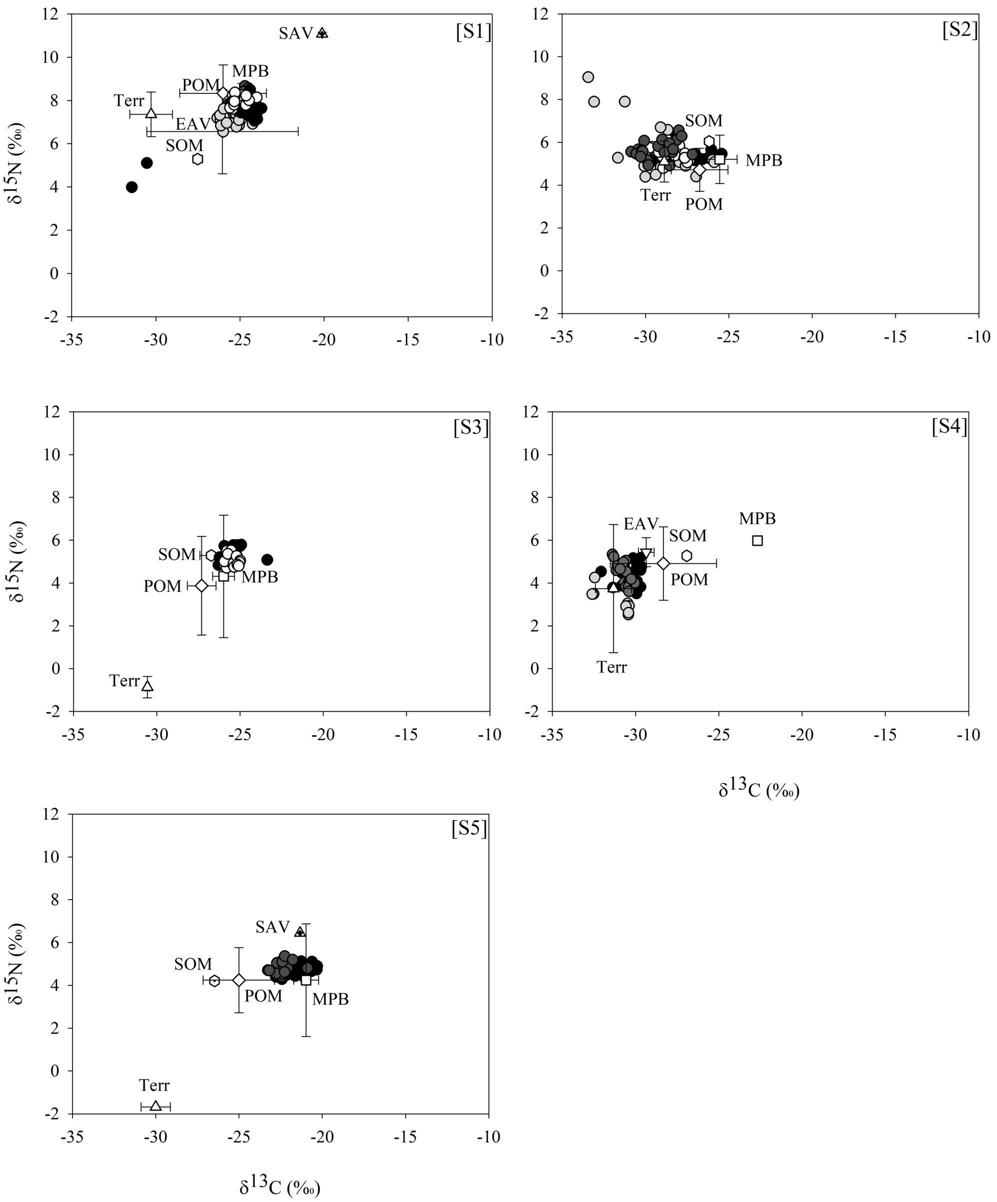

FIGURE 3 Average (± SD) δ^{13}C and δ^{15}N values of *Corbicula fluminea* (black), *Anodonta anatina* (light grey), *Potomida littoralis* (white), and *Unio delphinus* (dark grey) adjusted for one trophic level fractionation (+0.47‰ for δ^{13}C, +3.4‰ for δ^{15}N) and their potential organic matter sources. These sources include microphytobenthos (MPB), sediment organic matter (SOM), particulate organic matter (POM), terrestrial-derived organic matter (Terr), emergent (EAV) and submerged aquatic vegetation (SAV) at five sites (S1, S2, S3, S4, and S5) in the Tua basin. Error bars represent one standard deviation

consequence of reduced food availability, as consumers are forced to feed on less profitable food sources, hence widening their trophic niche (Rossi et al., 2015; Calizza et al., 2017). Although phytoplankton densities were not determined during the present study, bivalves relied on refractory food sources such as detritus derived from vascular plants across sites, suggesting that phytoplankton availability may have been low (Dias et al., 2014; Dias et al., 2016). Another possible explanation for the differences between studies may be related to the history of invasion. In the Tua basin, the invasion of *C. fluminea* occurred less than 15 years ago (Sousa et al., 2012). It is possible that in 2012 (Novais, Dias & Sousa, 2016) the process of invasion was in its early stages, and reducing interspecific competition for food sources with native freshwater mussels could have acted as an additional mechanism contributing to the successful invasion of *C. fluminea*. In fact, narrowing the trophic niche is traditionally proposed as a key process for reducing interspecific dietary overlap (Klopfer & MacArthur, 1961; Case, 1981). The expansion of *C. fluminea* within the Tua basin, which occurred in an upstream direction, may also be indicative of this behaviour. Except for *P. littoralis*, the trophic niche overlap between the invasive bivalve and the native freshwater mussels decreased upstream. Thus, further studies are needed to investigate the influence of food availability (quantity and quality) on the feeding behaviour of native freshwater mussels living in sympatry with *C. fluminea* and to understand how the history of invasion may influence interspecific trophic interactions.

The results of the dual-isotope mixing model showed that all species assimilated different proportions of the same sources, which included MPB, SOM, and detritus derived from vascular plants, but the relative proportion of each source varied between sites. Although the relative abundance of each food source was not determined during this study, intraspecific variability in the proportions of the sources assimilated between sites may be related to microhabitat

TABLE 4 Proportion of each food source in the freshwater bivalves collected at five sites (S1, S2, S3, S4, and S5) in the Tua basin (south-west Europe)

Site	Species	MPB	SOM	POM	Terr	EAV	SAV
S1	*Corbicula fluminea*	70 (46–83)	-	9 (0–30)	-	21 (11–31)	-
	Anodonta anatina	48 (29–69)	-	20 (1–38)	-	33 (19–46)	-
	Potomida littoralis	74 (59–90)	-	14 (1–28)	-	11 (3–19)	-
	Unio delphinus	71 (54–86)	-	15 (1–30)	-	14 (6–22)	-
S2	*Corbicula fluminea*	8 (0–22)	40 (26–51)	22 (7–34)	30 (17–42)	-	-
	Anodonta anatina	-	-	-	-	-	-
	Potomida littoralis	1 (0–11)	27 (10–46)	4 (0–18)	64 (41–76)	-	-
	Unio delphinus	1 (0–12)	26 (7–42)	1 (0–13)	66 (52–79)	-	-
S3	*Corbicula fluminea*	4 (0–15)	91 (80–99)	1 (0–9)	-	-	-
	Potomida littoralis	6 (0–12)	87 (79–93)	7 (0–13)	-	-	-
S4	*Corbicula fluminea*	-	-	6 (0–23)	34 (23–47)	56 (38–72)	-
	Anodonta anatina	-	-	4 (0–42)	57 (34–83)	25 (0–47)	-
	Unio delphinus	-	-	5 (0–22)	41 (28–54)	50 (33–67)	-
S5	*Corbicula fluminea*	39 (27–51)	-	12 (2–24)	-	-	49 (36–59)
	Unio delphinus	22 (13–34)	-	32 (24–42)	-	-	45 (34–54)

Note: Food sources included in the model were microphytobenthos (MPB), sediment organic matter (SOM), particulate organic matter (POM), terrestrial-derived organic matter (Terr), emergent (EAV), and submerged aquatic vegetation (SAV). The upper value indicates the most likely value (mode) and the ranges indicate the 95% Bayesian credibility intervals. Where no value is shown, sources were not included as end members in the model.

characteristics, to source availability, or both. For instance, *U. delphinus* and *C. fluminea* at site 5, were usually found associated with patches of SAV. The source with the highest contribution to the biomass of these species at this site was the detritus from SAV. Thus, the assimilation of this source at site 5 may be related to its increased availability as a consequence of microhabitat use. In addition, some individuals of *C. fluminea* and *A. anatina* at sites 1 and 2, respectively, displayed lower δ^{13}C values than those from the sources sampled. These differences may be related to a certain heterogeneity in the microhabitat characteristics within the same site (not measured in this study), which can promote differences in source availability (quantity, type, or both).

Previous studies have shown that in large rivers, where phytoplankton abundance is high, freshwater bivalves feed almost exclusively on this source (Raikow & Hamilton, 2001; Vaughn & Hakenkamp, 2001), whereas in oligotrophic systems, food sources such as bacteria, detritus, or others, can be used as an alternative to meet their nutritional requirements (Vaughn, Nichols & Spooner, 2008). In a study conducted in the Minho River estuary (north-west Iberian Peninsula), it was observed that *C. fluminea* increases the assimilation of phytoplankton when its availability is higher, spatially and temporally, thus showing its opportunistic feeding behaviour (Dias et al., 2014; Dias et al., 2016). It was also found that when phytoplankton availability was lower, *C. fluminea* relied on sources in the sediments (MPB and SOM) and on detritus derived from vascular plants (Dias et al., 2014; Dias et al., 2016), indicating that this species is also a generalist consumer. The mechanisms responsible for this adaptability may be related to the flexible feeding behaviour of freshwater bivalves. Although filter-feeding is the main mechanism for obtaining food (Dillon, 2000), freshwater mussels and *C. fluminea* can supplement suspension feeding in the water column by feeding on food in the sediments, through filtration of interstitial water or by pedal-feeding (Lopez & Holopainen, 1987; Way et al., 1990; Reid et al., 1992; Hakenkamp & Palmer, 1999; Raikow & Hamilton, 2001; Vaughn & Hakenkamp, 2001). Another possible mechanism to obtain food from the sediment includes filtration of resuspended SOM, as suggested for some marine filter-feeding bivalves (Watanabe et al., 2009; Dias et al., 2019).

Although this study shows that freshwater mussels have flexible diets, *P. littoralis* seemed to rely more on food available in the sediments than in the water column. This is further supported by the lower trophic niche width recorded for this species when compared with the other species analysed. Native freshwater mussels tend to be epibenthic, living half-buried in the sediment in lotic and lentic habitats (Reis, 2006; Araujo et al., 2009). However, *P. littoralis* is usually found more exposed to lotic conditions than any of the other freshwater bivalves studied here. Therefore, and to avoid shear stress, it is mostly found under the cover of rocks, between tree roots, or almost completely buried in the sediment (Haas, 1917; Reis, 2006; Araujo et al., 2009). Lotic environments have high variability in the water flow conditions, which can influence the behaviour of freshwater mussels. For example, the mussel *Fusconaia flava* has a sedentary behaviour and may be able to avoid displacement by burrowing deeper into the sediment when water flow increases (Allen & Vaughn, 2009). *Potomida littoralis* may be able to behave similarly, preventing its displacement during high water flow while facilitating its access to food in the sediments. Similarly, *Corbicula* *fluminea* usually lives buried in the sediment in lotic and lentic habitats (Araujo, Moreno & Ramos, 1993; Sousa, Antunes & Guilhermino, 2006; Sousa, Antunes & Guilhermino, 2007; Sousa, Antunes & Guilhermino, 2008; Sousa et al., 2008a; Sousa et al., 2008b). Thus, because *P. littoralis* exhibited the lowest trophic niche width, the highest probability values of *C. fluminea* to overlap with its trophic niche, as well as its similar microhabitat use, may suggest that this native species is at a higher risk of being adversely affected by interspecific competition with *C. fluminea*.

Although there are no data on freshwater mussels before the invasion of *C. fluminea*, nor any studies elsewhere showing their decline caused by competition for food with *C. fluminea*, this IAS may be considered a superior competitor for food resources than the native mussels. In addition to displaying flexible feeding strategies, *C. fluminea* also has high filtration and assimilation rates per individual (Hakenkamp & Palmer, 1999; Vaughn & Hakenkamp, 2001; Werner & Rothhaupt, 2008). Moreover, *C. fluminea* can achieve high densities in those ecosystems invaded (Sousa, Antunes & Guilhermino, 2008; Ilarri & Sousa, 2012). The combination of these characteristics can intensify the competition for food with native freshwater mussels when food is limited; this situation is expected to be more prevalent in oligotrophic systems, where phytoplankton availability is low (Ferreira-Rodríguez, Sousa & Pardo, 2018).

Several efforts to develop conservation strategies for native freshwater mussels have prioritized species and populations to preserve (Geist, 2010; Strayer, 2017). Two of the native species studied here have poor conservation status: *P. littoralis* is categorized as Endangered and *U. delphinus* as Near Threatened by the International Union for Conservation of Nature (IUCN, 2017). Given the global widespread distribution of *C. fluminea*, the implementation of management measures devoted to the control or even the eradication of this IAS should be a conservation priority given its potential for competition with highly threatened native freshwater mussels such as *P. littoralis* in this study area. In addition, oligotrophic ecosystems should be prioritized, given the lower availability of food sources for freshwater mussels that live in sympatry with invasive bivalves.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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