

# Inter- and intraspecific variation of carbon and nitrogen stable isotope ratios in freshwater bivalves

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**Abstract** Freshwater bivalves provide important ecosystem functions and services, yet many of their ecological traits such as feeding mechanisms and resource use are largely ignored. In this study, we aimed to evaluate the potential overlap in resource use by bivalve species living in sympatry in European freshwater ecosystems. This was accomplished by analyzing the stable isotope ratios of carbon (C) and nitrogen (N) values of six bivalve species (five native species plus the invasive species *Corbicula fluminea*) in six distinct aquatic ecosystems. Results showed significant inter- and intraspecific differences in both stable isotope ratios. The interspecific variability suggests differences in the food sources consumed, which can be related to differences in feeding behavior. At the intraspecific level, there was a gradient in the stable isotope ratios from the oligotrophic River

Paiva (<sup>15</sup>N-depleted and <sup>13</sup>C-enriched) to the eutrophic Mira Lagoon (<sup>15</sup>N-enriched and <sup>13</sup>C-depleted), suggesting a change in the resources used from benthic to pelagic food sources, respectively, and/or differences in the stable isotopic baseline in each ecosystem. Thus, flexible feeding strategies combined with size selectivity may decrease the possible competition for food sources by native and invasive species living in sympatry.

**Keywords** Bivalves · *Corbicula fluminea* · Freshwater ecosystems · Invasive and native species · Stable isotopes

## Introduction

Reports describing the decline of freshwater bivalves have increased in recent years, being these invertebrates recognized today as one of the most threatened faunistic groups worldwide (Strayer et al., 2004; Régner et al., 2009). These declines may have important consequences at all levels of ecological organization, from individuals to ecosystems (Vaughn & Taylor, 1999; Vaughn et al., 2008; Sousa et al., 2011). At the ecosystem level, the decline or even disappearance of these species, along with possible replacements by invasive bivalves, may lead to changes in trophic relationships and food web dynamics, as these organisms are important primary consumers in many aquatic ecosystems (Strayer, 2010;

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Lopes-Lima et al., 2014a; Sousa et al., 2014). In addition, these organisms can dominate the benthic biomass in many rivers and lakes exerting a large influence in nutrient cycling through the selective uptake and assimilation of carbon (C) and nitrogen (N) during the filtration process (Dame, 1996; Vaughn et al., 2008). However, their trophic role remains highly speculative and further studies encompassing new methodologies are needed to assess the possible differences in resource use between bivalve species, including native and invasive species living in sympatry (e.g., Hakenkamp & Palmer, 1999; Vaughn & Hakenkamp, 2001).

Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope analysis is a powerful tool to characterize energy flow through aquatic food webs (West et al., 2006; Layman et al., 2012). This technique is based on the relationship between the isotopic composition of organic matter (OM) in the ecosystem and the isotopic composition of consumers' tissues that incorporate this OM in their structural components and energy reserves (Peterson & Fry, 1987). Thus, the stable isotope ratio of a consumer reflects its diet, with a trophic fractionation between 3 and 5‰ for  $\delta^{15}\text{N}$  (Minagawa & Wada, 1984) and 0 and 1‰ for  $\delta^{13}\text{C}$  (DeNiro & Epstein, 1978; Zanden & Rasmussen, 2001; McCutchan et al., 2003) per trophic level. Although stable isotope analysis has emerged as one of the most useful methods for examining the structure and dynamics of food webs (Layman et al., 2012), this technique has been rarely applied to sympatric populations of freshwater bivalves (but see Atkinson et al., 2010).

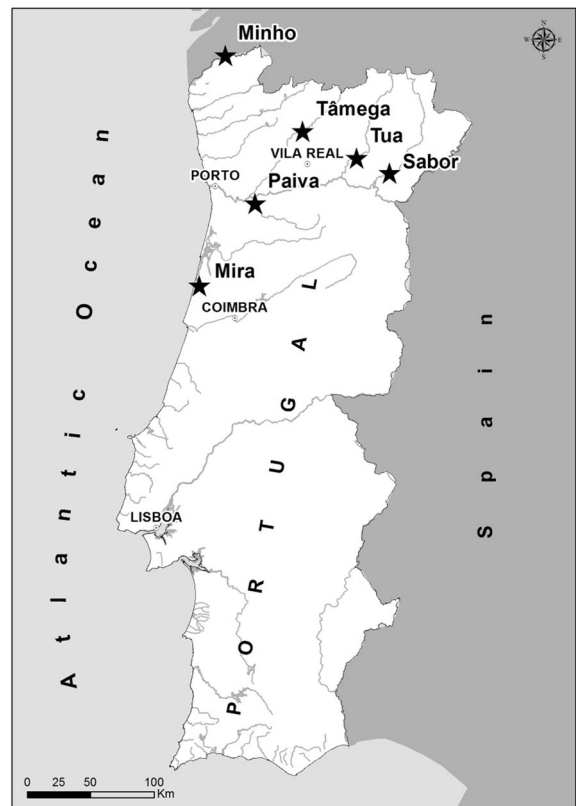
In this study, we aimed to evaluate the potential overlap in resource use by bivalve species living in sympatry in European freshwater ecosystems, by analyzing the interspecific variability in their C and N stable isotope ratios. Special attention was given to the possible overlap between native bivalve species [*Anodonta anatina* (Linnaeus, 1758), *Anodonta cygnea* (Linnaeus, 1758), *Pisidium amnicum* (Müller, 1774), *Potomida littoralis* (Cuvier, 1798), and *Unio delphinus* (Spengler, 1793)], and one of the most pervasive invasive species in European aquatic ecosystems, the Asian clam *Corbicula fluminea* (Müller, 1774) (Sousa et al., 2008a). Also, we aimed to evaluate the intraspecific variability in the C and N stable isotope ratios in ecosystems subjected to different environmental conditions.

## Materials and methods

### Study area

The bivalve species used in this study were collected in six different aquatic ecosystems, Mira Lagoon and Rivers Minho, Paiva, Tâmega, Tua and Sabor, located in the north and center of Portugal (see Fig. 1 for sites location). The climate of the studied ecosystems reflects the proximity to the Atlantic Ocean and altitude, ranging from temperate near the coast to Mediterranean more inland (Oliveira et al., 2012).

The Mira Lagoon is a shallow freshwater lagoon (depth ranging from a few centimeters to 2 m), with an area of 18 ha and is distant 5 km from the sea. This lagoon is inserted in a mixed urban/agricultural/forest zone and is used mainly for recreation (sport fisheries and tourism), connected through a series of man-made



**Fig. 1** Map showing the six sampling sites: Mira Lagoon (40°26′28.45″N; 08°45′09.45″W); River Minho (42°04′36.68″N; 08°30′52.93″W); River Paiva (41°01′37.11″N; 08°14′22.60″W); River Tâmega (41°32′38.18″N; 07°47′07.36″W); River Tua (41°20′47.27″N; 07°16′59.10″W) and River Sabor (41°14′24.71″N; 06°58′01.90″W)

canals to the River Vouga. The most significant pressures are related to sedimentation, agriculture, and domestic and urban contamination being considered a eutrophic system, and some studies revealed the presence of toxic cyanobacteria (Garcia, 2001). The River Minho covers a total basin area of 17,080 km<sup>2</sup> and has a total length of 300 km with a mean annual freshwater discharge of 300 m<sup>3</sup> s<sup>-1</sup> (Ferreira et al., 2003; Sousa et al., 2005). This river is considered a low impacted system, although there are some problems related to the introduction of invasive species (Sousa et al., 2008b). The River Tâmega has a total basin area of 3309 km<sup>2</sup> and a total length of 140 km. This river receives some organic pollution derived from agriculture and industry, and is usually considered a eutrophic system (Sousa et al., 2012). The River Tua has a total basin area of 3813 km<sup>2</sup> and a total length of 106 km. This river is in good ecological conditions throughout most of its length, mainly due to low levels of human pressure, although there is some evidence of organic pollution in downstream areas (Ferreiro, 2007; Sousa et al., 2012). The River Sabor has a total basin area of 3868 km<sup>2</sup> and a total length of 112 km long. This river is in good environmental condition, although in a very few stretches there are some evidences of organic pollution (Sousa et al., 2012); presently, the downstream area of the basin is flooded by the reservoir of the Baixo Sabor Hydroelectric Infrastructure. The River Paiva is entirely located in Portugal with a total basin area of 795 km<sup>2</sup> and a total length of 108 km. In general, its water quality is excellent with oligotrophic conditions in its entire extension and it is usually classified as one of the pristine rivers in Portugal (Sousa et al., 2012, 2013).

### Sampling strategy

Sampling was conducted in September 2012, always during the morning. Temperature (°C), conductivity (µS cm<sup>-1</sup>), dissolved oxygen (mg l<sup>-1</sup>), and pH were measured in situ using a multi-parametric probe YSI 6820. Water samples were collected to determine the concentration (mg l<sup>-1</sup>) of nitrites, nitrates, ammonia, and phosphates using colorimetric methods (following Sousa et al., 2008b).

Whenever possible, twelve individuals of each species were collected at each sampling site. In the Mira lagoon, only six specimens of *C. fluminea* and *U. delphinus* were collected, and in the River Paiva only

two specimens of *U. delphinus* were collected. It was not possible to collect organisms from the six different species in sympatry in the six sampled sites due to their absence or very low abundance; however, it was possible to collect *C. fluminea* in all sites (see Table 1). At each site, bivalves were hand collected at a water depth lower than 1 m and within a 50 m reach subjected to similar abiotic conditions. The shell length of all specimens was measured to the nearest mm (a proxy for organism age; Howard et al., 2005): *A. anatina* 95.7 ± 15.8 mm, *A. cygnea* 140.8 ± 6.8 mm, *C. fluminea* 27.2 ± 6.2 mm, *P. amnicum* 7.9 ± 1.3 mm, *P. littoralis* 71.7 ± 8.7 mm, and *U. delphinus* 65.5 ± 18.3 mm. After collection of tissue samples (see below), the specimens belonging to native species were carefully returned to their habitat in the same position.

For stable isotope analysis, a small piece of foot-muscle tissue was removed from all specimens, dried in an oven at 60°C for 48 h, and ground to a fine powder. Stable isotope ratios were measured using a Thermo Scientific Delta V Advantage IRMS via a Conflo IV interface (Interdisciplinary Centre of Marine and Environmental Research—CIIMAR/CIMAR—University of Porto). Stable isotope ratios are reported in δ notation, δX:  $\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3$ , where X is the C or N stable isotope, R is the ratio of heavy:light stable isotopes, and Pee Dee Belemnite and air are standards for δ<sup>13</sup>C and δ<sup>15</sup>N, respectively. The analytical error, the mean standard deviation (SD) of replicate reference material, was ±0.1‰ for δ<sup>13</sup>C and δ<sup>15</sup>N. To control for sample processing quality, we did not include those samples with a SD between replicates (i.e., two subsamples of the same sample) >0.2‰ δ<sup>13</sup>C and/or δ<sup>15</sup>N in subsequent data analyses. The δ<sup>13</sup>C values were corrected for lipid content because lipids are more depleted in <sup>13</sup>C compared to protein and carbohydrates (DeNiro & Epstein, 1977). Variability in tissue lipid content can bias bulk tissue δ<sup>13</sup>C values; therefore, data were corrected for lipid content using tissue C:N following the mass balance correction proposed by Logan et al. (2008, Eq. 2).

### Data analysis

All 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).

**Table 1** Results of PERMANOVA test for differences of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between different species in each site (A) and for each species between sites (B)

A			B		
	<i>T</i>	<i>P</i> value		<i>T</i>	<i>P</i> value
Mira Lagoon			<i>Corbicula fluminea</i>		
<i>A. cygnea</i> versus <i>C. fluminea</i>	5.488	0.0001	Mira Lagoon versus River Minho	44.519	0.0001
<i>A. cygnea</i> versus <i>U. delphinus</i>	3.764	0.0002	Mira Lagoon versus River Paiva	63.696	0.0001
<i>C. fluminea</i> versus <i>U. delphinus</i>	3.247	0.009	Mira Lagoon versus River Sabor	36.533	0.0002
River Minho			Mira Lagoon versus River Tâmega	26.181	0.0001
<i>C. fluminea</i> versus <i>U. delphinus</i>	2.875	0.0028	Mira Lagoon versus River Tua	29.063	0.0002
<i>C. fluminea</i> versus <i>A. anatina</i>	4.928	0.0001	River Minho versus River Paiva	57.959	0.0001
<i>C. fluminea</i> versus <i>P. amnicum</i>	14.517	0.0001	River Minho versus River Sabor	13.403	0.0001
<i>C. fluminea</i> versus <i>P. littoralis</i>	5.792	0.0001	River Minho versus River Tâmega	18.424	0.0001
<i>U. delphinus</i> versus <i>A. anatina</i>	6.976	0.0001	River Minho versus River Tua	21.386	0.0001
<i>U. delphinus</i> versus <i>P. amnicum</i>	14.861	0.0001	River Paiva versus River Sabor	38.464	0.0001
<i>U. delphinus</i> versus <i>P. littoralis</i>	3.992	0.0001	River Paiva versus River Tâmega	44.569	0.0001
<i>A. anatina</i> versus <i>P. amnicum</i>	12.612	0.0001	River Paiva versus River Tua	33.439	0.0001
<i>A. anatina</i> versus <i>P. littoralis</i>	8.786	0.0001	River Sabor versus River Tâmega	13.300	0.0001
<i>P. amnicum</i> versus <i>P. littoralis</i>	13.512	0.0001	River Sabor versus River Tua	11.406	0.0001
River Paiva			River Tâmega versus River Tua	80.265	0.0001
<i>C. fluminea</i> versus <i>U. delphinus</i>	4.790	0.0005	<i>Anodonta anatina</i>		
<i>C. fluminea</i> versus <i>A. anatina</i>	2.445	0.0054	River Minho versus River Paiva	44.044	0.0001
<i>U. delphinus</i> versus <i>A. anatina</i>	3.069	0.0016	River Minho versus River Tâmega	8.693	0.0001
River Sabor			River Minho versus River Tua	21.981	0.0001
<i>C. fluminea</i> versus <i>U. delphinus</i>	6.515	0.0001	River Paiva versus River Tâmega	46.146	0.0001
<i>C. fluminea</i> versus <i>P. littoralis</i>	5.252	0.0001	River Paiva versus River Tua	46.564	0.0001
<i>U. delphinus</i> versus <i>P. littoralis</i>	2.903	0.0006	River Tâmega versus River Tua	14.519	0.0001
River Tâmega			<i>Potomida littoralis</i>		
<i>C. fluminea</i> versus <i>A. anatina</i>	13.440	0.0001	River Minho versus River Sabor	12.702	0.0001
River Tua			River Minho versus River Tua	18.286	0.0001
<i>C. fluminea</i> versus <i>U. delphinus</i>	11.557	0.0001	River Sabor versus River Tua	28.094	0.0001
<i>C. fluminea</i> versus <i>A. anatina</i>	10.902	0.0001	<i>Unio delphinus</i>		
<i>C. fluminea</i> versus <i>P. littoralis</i>	8.326	0.0001	Mira Lagoon versus River Minho	17.443	0.0001
<i>U. delphinus</i> versus <i>A. anatina</i>	2.694	0.0034	Mira Lagoon versus River Paiva	17.112	0.0001
<i>U. delphinus</i> versus <i>P. littoralis</i>	5.816	0.0001	Mira Lagoon versus River Sabor	17.112	0.0001
<i>A. anatina</i> versus <i>P. littoralis</i>	4.539	0.0001	Mira Lagoon versus River Tua	13.392	0.0002
			River Minho versus River Paiva	31.675	0.0001
			River Minho versus River Sabor	11.364	0.0001
			River Minho versus River Tua	17.152	0.0001
			River Paiva versus River Sabor	24.687	0.0001
			River Paiva versus River Tua	27.816	0.0001
			River Sabor versus River Tua	22.002	0.0001

A principal component analysis (PCA) was used to detect differences on surveyed sites based on abiotic characteristics. To test for possible differences in the

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between species and sites, we used a two-way PERMANOVA (type-III) in a two-way crossed design, with species (six levels:

*A. anatina*, *A. cygnea*, *C. fluminea*, *P. amnicum*, *P. littoralis*, and *U. delphinus*) and sites (six levels: Mira Lagoon, River Minho, River Paiva, River Tâmega, River Tua, and River Sabor) as fixed factors. PERMANOVA tests the simultaneous response of one or more variables to one or more factors in an ANOVA experimental design on the basis of any distance measure, using permutation methods (Anderson, 2001). In all PERMANOVA tests, 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. Two-way PERMANOVA pairwise comparisons were also performed for all PERMANOVA tests.

Prior to PCA and PERMANOVA ordination analyses, all variables were normalized without data transformation, and resemble matrices based on the Euclidean distances were calculated (Clarke & Warwick, 2001).

## Results

The PCA analysis revealed a clear separation between the Mira Lagoon and the remaining sites (Rivers Minho, Paiva, Sabor, Tâmega, and Tua) (Fig. 2). From the projection of the first axis (74.9% of variation explained), the most important abiotic factors were nutrients and conductivity (for the negative side), and oxygen (for the positive side). Along the second axis (14.8% of variation explained), the most important abiotic factors were nitrates (for the negative side) and oxygen, temperature, and pH (for the positive side). The Mira Lagoon showed high values of conductivity ( $250.00 \mu\text{S cm}^{-1}$ ), ammonia ( $0.19 \text{ mg l}^{-1}$ ), nitrites ( $0.08 \text{ mg l}^{-1}$ ), nitrates ( $2.50 \text{ mg l}^{-1}$ ), and phosphates ( $0.25 \text{ mg l}^{-1}$ ), and lower values of oxygen ( $8.10 \text{ mg l}^{-1}$ ). The River Paiva presented the lowest values for the majority of the abiotic factors analyzed (conductivity:  $56.50 \mu\text{S cm}^{-1}$ , ammonia:  $0.01 \text{ mg l}^{-1}$ , nitrites:  $0.01 \text{ mg l}^{-1}$ , nitrates:  $0.60 \text{ mg l}^{-1}$ , phosphates:  $0.01 \text{ mg l}^{-1}$ , and temperature:  $21.20^\circ\text{C}$ ), with the exception of oxygen with  $9.02 \text{ mg l}^{-1}$ . The Rivers Minho, Tâmega, Tua, and Sabor presented intermediate values, with the exception of oxygen which was higher in River Minho with  $9.12 \text{ mg l}^{-1}$ , and temperature which was higher on Rivers Tua and Tâmega with  $24.30^\circ\text{C}$ .

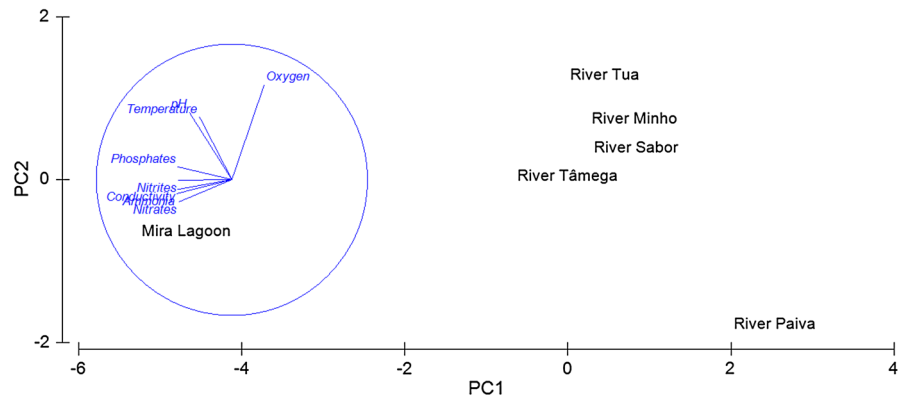
There were significant differences in the interaction species  $\times$  site (Pseudo- $F = 32.25$ ;  $P < 0.001$ ). Pairwise comparisons showed significant differences in the isotope ratios across all species at each site (Table 1A) and for each species in the different sites (Table 1B). There were significant differences between species in each ecosystem. Overall, *C. fluminea* presented the highest  $\delta^{13}\text{C}$  values, except in the River Minho, where *P. amnicum* was the more  $^{13}\text{C}$ -enriched species (Fig. 3; Table 2). The Unionidae species presented the lowest  $\delta^{13}\text{C}$  values: *A. cygnea* and *A. anatina* in the Mira Lagoon and Rivers Minho and Tâmega, and *U. delphinus* in the Rivers Tua, Sabor, and Paiva. *Corbicula fluminea* was also the species presenting the highest  $\delta^{15}\text{N}$  values in each ecosystem, except in the Rivers Minho, Paiva, and Tua, where *U. delphinus* were more  $^{15}\text{N}$ -enriched (Fig. 3; Table 2).

Specimens collected in the Mira Lagoon and River Paiva presented the more extreme values (Fig. 3; Table 2). In fact, the bivalves collected in the Mira Lagoon were the most enriched in  $^{15}\text{N}$  ( $\delta^{15}\text{N}$ :  $15.5 \pm 0.5\text{‰}$ ) and depleted in  $^{13}\text{C}$  ( $\delta^{13}\text{C}$ :  $-28.0 \pm 0.6\text{‰}$ ), and in the River Paiva the most depleted in  $^{15}\text{N}$  ( $\delta^{15}\text{N}$ :  $7.9 \pm 0.4\text{‰}$ ) and enriched in  $^{13}\text{C}$  ( $\delta^{13}\text{C}$ :  $-21.2 \pm 0.4\text{‰}$ ) (Fig. 3; Table 2). Individuals of the different species collected in the Rivers Minho, Tâmega, Tua, and Sabor presented intermediate values in relation to those observed in the Mira Lagoon and River Paiva (Fig. 3; Table 2).

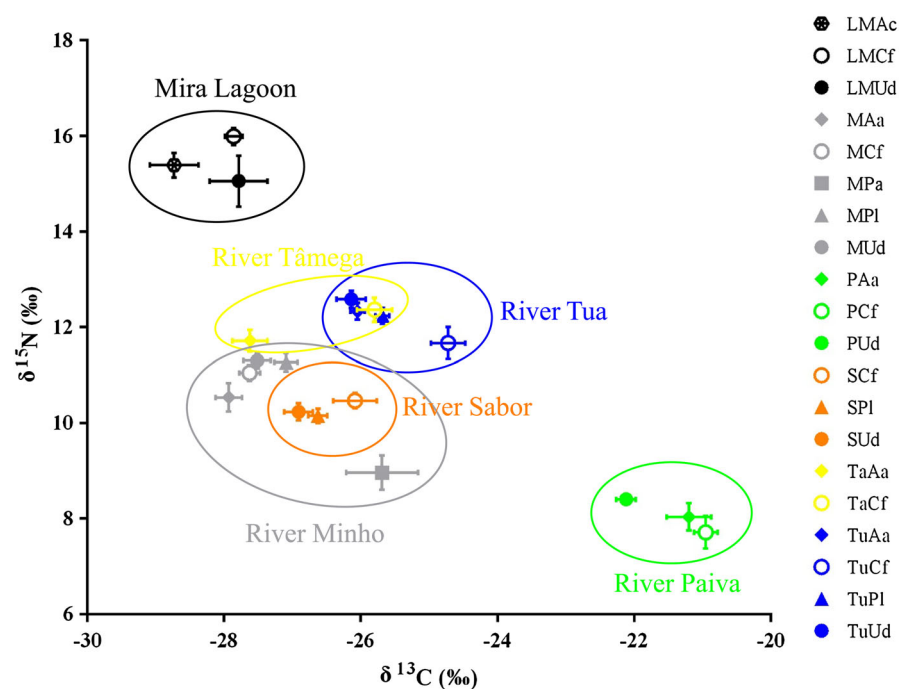
## Discussion

Significant interspecific differences were observed in the stable isotope ratios of C and N of freshwater bivalves collected within ecosystems. Although with some exceptions, *C. fluminea* was the species presenting the highest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in each ecosystem, indicating the contribution of  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enriched food sources to their biomass, which can include benthic phytoplankton (Fry, 1991; Cloern et al., 2002; Hoffman & Bronk, 2006) or other microbial enriched materials (Angradi, 1994; Goedkoop et al., 2006). The other bivalve species were in general more  $^{13}\text{C}$ - and  $^{15}\text{N}$ -depleted than *C. fluminea*, indicating the consumption of more  $^{13}\text{C}$ - and  $^{15}\text{N}$ -depleted food sources such as pelagic OM. Organic matter in suspension and in the sediments can include

**Fig. 2** Principal Component Analysis (PCA) showing the plotting of the six sampling sites (Mira Lagoon and Rivers Minho, Paiva, Sabor, Tâmega, and Tua). PC1 explained 74.9% of overall variation and PC2 14.8%



**Fig. 3** Tissue  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of all species (*Cf*, *C. fluminea*; *Aa*, *A. anatina*; *Pl*, *P. littoralis*; *Ud*, *U. delphinus*; *Pa*, *P. amnicum* and *Ac*, *A. cygnea*) at all sampling sites (*LM* Mira Lagoon, *M* River Minho, *P* River Paiva, *S* River Sabor, *Ta* River Tâmega, and *Tu* River Tua). The ovals are grouping the species from each sampling site



phytoplankton, bacteria, nanoflagellates, and detritus with different origins. Although the most likely food sources to the species analyzed were not sampled, benthic food sources tend to be more  $^{13}\text{C}$ -enriched due to the existence of a diffusive boundary layer at the sediment–water interface that reduces isotopic fractionation (France, 1995). The variability in the stable isotope values of freshwater bivalves within ecosystems suggests that they might be using distinct food sources or, in some cases, different proportions of the same food sources; nonetheless, differences in feeding selectivity may also explain some of the observed

discrepancies. Silverman et al. (1997) have hypothesized that differences in the number and spacing of cilia on the gills might allow distinct species to specialize on different particle types or sizes. *Corbicula fluminea* is usually assumed to be a non-selective suspension feeder, but recent studies indicate that this species selectively feed on small-sized particles ( $\leq 1\text{--}10\ \mu\text{m}$ ) such as picoautotrophs (Way et al., 1990; Silverman et al., 1995; Atkinson et al., 2011). Contrary, unionids can feed on larger particles ( $< 20\ \mu\text{m}$ ; Vaughn et al., 2008) such as nanoeukaryotes (Atkinson et al., 2011). Therefore, *C. fluminea*

**Table 2** Mean  $\pm$  SD (‰) values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of all species at all sampling sites

	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Mira Lagoon		
<i>A. cygnea</i>	$-28.7 \pm 0.4$	$15.4 \pm 0.3$
<i>U. delphinus</i>	$-27.8 \pm 0.4$	$15.1 \pm 0.5$
<i>C. fluminea</i>	$-27.9 \pm 0.1$	$16.0 \pm 0.2$
River Minho		
<i>A. anatina</i>	$-27.9 \pm 0.2$	$10.5 \pm 0.3$
<i>P. littoralis</i>	$-27.1 \pm 0.2$	$11.3 \pm 0.2$
<i>U. delphinus</i>	$-27.5 \pm 0.2$	$11.3 \pm 0.2$
<i>P. amnicum</i>	$-25.7 \pm 0.5$	$9.0 \pm 0.4$
<i>C. fluminea</i>	$-27.6 \pm 0.2$	$11.0 \pm 0.2$
River Paiva		
<i>A. anatina</i>	$-21.2 \pm 0.3$	$8.0 \pm 0.3$
<i>U. delphinus</i>	$-22.1 \pm 0.1$	$8.4 \pm 0.1$
<i>C. fluminea</i>	$-21.0 \pm 0.2$	$7.7 \pm 0.3$
River Sabor		
<i>U. delphinus</i>	$-26.9 \pm 0.2$	$10.2 \pm 0.2$
<i>P. littoralis</i>	$-26.6 \pm 0.1$	$10.2 \pm 0.2$
<i>C. fluminea</i>	$-26.1 \pm 0.3$	$10.5 \pm 0.2$
River Tâmega		
<i>A. anatina</i>	$-27.6 \pm 0.3$	$11.7 \pm 0.2$
<i>C. fluminea</i>	$-25.8 \pm 0.3$	$12.4 \pm 0.3$
River Tua		
<i>A. anatina</i>	$-26.1 \pm 0.1$	$12.3 \pm 0.2$
<i>P. littoralis</i>	$-25.7 \pm 0.1$	$12.2 \pm 0.2$
<i>U. delphinus</i>	$-26.1 \pm 0.2$	$12.6 \pm 0.2$
<i>C. fluminea</i>	$-24.7 \pm 0.3$	$11.7 \pm 0.3$

might be more efficient at capturing smaller particles due to the close spacing of their cirri when compared to unionid mussels (Way et al., 1989). Additionally, other factors may contribute to the observed interspecific differences such as pre-sorting of food particles using the labial palps (Galbraith et al., 2009), differential assimilation efficiency (Atkinson et al., 2010), or morphological and functional adaptations in response to competition for food. There were also significant intraspecific differences in the stable isotope ratios of C and N between ecosystems. Overall, there was a gradient from low  $\delta^{15}\text{N}$  and high  $\delta^{13}\text{C}$  values in the River Paiva to high  $\delta^{15}\text{N}$  and low  $\delta^{13}\text{C}$  values in the Mira Lagoon, which suggests a change in the resources used from benthic energy sources (e.g., periphyton, benthic phytoplankton, or other OM in the sediment) in the oligotrophic River Paiva to pelagic energy sources

(e.g., phytoplankton) in the eutrophic Mira Lagoon (Sousa et al., 2013; Lopes-Lima et al., 2014b). Previous studies showed that in large productive rivers, where phytoplankton is abundant, bivalves feed almost exclusively on this source (Thorp et al., 1998; Raikow & Hamilton, 2001; Vaughn & Hakenkamp, 2001), while in oligotrophic systems, where phytoplankton growth is limited due to low concentration of nutrients or to reduced light incidence due to the presence of dense riparian vegetation, other sources such as bacteria, detritus, or others can be used as an alternative to meet nutritional requirements (Boltovskoy et al., 1995; Vaughn et al., 2008, Dias et al., 2014). The mechanisms underlying this adaptability can be related to bivalves' flexible feeding behavior. Filter feeding is generally accepted as the main mechanism used by freshwater bivalves to obtain food (Dillon, 2000). However, some freshwater bivalves, including unionids, *Pisidium*, and *Corbicula* can supplement suspension feeding in the water column by feeding on OM in the sediments, through filtration of the 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; Dias et al., 2014). Differences in the stable isotope ratios of the primary consumers available in each ecosystem may also have influenced the intraspecific variability observed in the bivalves' stable isotope ratios. Factors such as the available inorganic pool (Cabana & Rasmussen, 1996; Finlay et al., 1999; Zanden & Rasmussen, 1999) and/or species composition (McNeely et al., 2006; Atkinson et al., 2009) influence the isotopic baseline in each ecosystem. Organic pollution could also contribute to the differences observed between ecosystems (Atkinson et al., 2014). Usually, nitrate from human and animal wastes are typically more  $^{15}\text{N}$ -enriched than other N sources, with  $\delta^{15}\text{N}$  values ranging from 9 to 25‰ (Kreitler & Browning, 1983; Rolston et al., 1996; McKinney et al., 2002). The sampled ecosystems followed a gradient of organic pollution with River Paiva presenting the lowest impact, whereas Mira Lagoon was the ecosystem subjected to the highest levels of organic pollution (Oliveira et al., 2012; Sousa et al., 2012, 2013). The  $\delta^{15}\text{N}$  values of the bivalves sampled also followed a gradient, with the lowest values observed in the River Paiva ( $\delta^{15}\text{N}$ :  $7.9 \pm 0.4\text{‰}$ ), and the highest in the Mira Lagoon ( $\delta^{15}\text{N}$ :  $15.5 \pm 0.5\text{‰}$ ).

Although this is the first study using European native and invasive bivalve species in sympatry, there are two major limitations to consider when interpreting these results. First, the lack of data regarding stable isotope ratios of the most likely food sources in each ecosystem impairs the identification and quantification of the main food sources used by the studied freshwater bivalve species. Thus, it is not possible to clarify if the differences observed between species were due to the consumption of different sources or to the consumption of different proportions of the same sources, and if the intraspecific variability was mainly related to differences in the isotopic baseline or to the available food sources. Second, this study was performed only in the summer, and feeding strategies may vary seasonally (Vaughn, 2010). Nonetheless, the results from this study suggest that freshwater bivalves have flexible feeding strategies, which allows them to adapt in order to feed according to the available resources. If the availability of phytoplankton decreases, they will be able to use sources available in the sediment or resuspended from the sediments. Also, the C and N stable isotope ratios of freshwater bivalves living in sympatry suggest that they use different food sources or at least different proportions of the same food sources, allowing partitioning of the food sources. Thus, the flexibility in accessing both pelagic and benthic OM, and the adaptation to filter particles with different sizes, may decrease the possible competition for food sources by native and invasive species living in sympatry. However, further studies are needed to identify which are the main food sources used by the different bivalve species, and to verify if invasive species compete with native species for the same resources. As bivalves are one of the most endangered faunal groups in freshwater ecosystems (Strayer et al., 2004; Sousa et al., 2008c, d; Lopes-Lima et al., 2014b), information regarding the resources they consume can be particularly informative for the conservation of native species and management of the impacts generated by invasive species such as *C. fluminea*.

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