

# Negative effects of *Corbicula fluminea* over native freshwater mussels

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**Abstract** The global decline of freshwater mussels is related with a great variety of factors, including the introduction of invasive species. However, the possible effects of other invasive bivalves, such as the Asian clam *Corbicula fluminea* (Müller, 1774), remain mainly unknown and highly speculative with very few manipulative experiments addressing this issue. In this study, field and laboratory experiments were conducted to assess the possible negative effects of *C. fluminea* on the native freshwater mussel *Unio*

*delphinus* Spengler, 1783. Growth, physiological condition, and the locomotor activity were assessed in *U. delphinus* at increasing *C. fluminea* density. *U. delphinus* exhibited lower growth, lower physiological condition, and higher locomotor activity at higher *C. fluminea* density, which may suggest that this unionid is negatively affected by *C. fluminea* and may be displaced to less favorable habitats. Although we were not able to establish the main mechanism(s) responsible for these results, possibilities include competition for food resources, competition for space which may result in mussel displacements and/or changes in microhabitat features as a result of bioturbation activities, and production of feces and pseudofeces by *C. fluminea*.

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## Introduction

Freshwater mussels (order Unionoida) are one of the most imperiled faunal groups on the planet, with high rates of extinctions (Lydeard et al., 2004; Strayer et al., 2004; Lopes-Lima et al., 2014, 2016). Their decline in spatial distribution and abundance has been related to a great variety of factors, which include habitat loss and fragmentation associated with dams or other type

of physical obstacles and water abstraction, dredging, and channelization, water quality degradation due to eutrophication or other types of pollution, overexploitation, and climate change (Vaughn & Taylor, 1999; Williams et al., 1998; Anthony & Downing, 2001; Patzner & Müller, 2001; Downing et al., 2010; Santos et al., 2015; Sousa et al., 2016; Zieritz et al., in press). In addition to the above-mentioned factors, during the last decades the introduction of invasive species has been also described as an important threat to freshwater mussel conservation. A great emphasis has been given to the negative effects imposed by invasive bivalves such as the zebra mussel *Dreissena polymorpha* (Pallas, 1771) on freshwater mussels (Hebert et al. 1991; Sousa et al., 2011a, 2014; Bódis et al., 2014; Krebs et al., 2015).

The Asian clam *Corbicula fluminea* (Müller, 1774) has also been cited as an important threat to freshwater mussel conservation (Leff et al., 1990). This species was first introduced in the United States of America in the first half of the twentieth century, then in South America in the late 1960s/beginning of the 1970s. It reached Europe in the late 1970s/beginning of the 1980s and North Africa in the last decade (for a recent review see Crespo et al., 2015). Therefore, in addition to the native range in Asia, nowadays the species is present in North and South America, North Africa, and in almost all European river basins, from the Iberian Peninsula in the Southwest to Ireland and UK in the North and to Bulgaria and Romania in the East (Crespo et al., 2015). Krumholz et al. (1969) were the first to suggest the possible negative impacts of *C. fluminea* on native freshwater mussels. Recent studies have shown its potential to impact both benthic and pelagic biological communities, including native bivalve populations (Isom, 1986; McMahon, 1991; Vaughn & Spooner, 2006; Ilarri et al., 2012, 2014; Novais et al., 2015, 2016b). Despite the potential threat of *C. fluminea* to aquatic ecosystems and their biodiversity, the impact on native bivalve species is understudied and there is a lack of consensus about the strength of interactions between *C. fluminea* and native bivalves living in sympatry (Strayer, 1999). Generally, the evidence is weak and most of the available information comes from field observations. This information is usually correlative or is inferred from the published literature, which mainly covers North American ecosystems (Clarke, 1988; Strayer, 1999). The reality is that a limited number of

manipulative experiments has established clear links between the presence of *C. fluminea* and the decline of freshwater bivalves; being most of these studies on sphaeriid clams (Strayer, 1999; Sousa et al., 2008c, d, 2011b). Some of the conclusions of these studies suggest that dense populations of *C. fluminea* may compete with freshwater bivalves for space and/or food resources (Strayer, 1999; Novais et al., 2016a). In addition, negative impacts on native freshwater mussels are also likely to occur through other mechanisms, such as possible ingestion of unionid sperm, glochidia, or newly metamorphosed juveniles by *C. fluminea*; massive mortalities of *C. fluminea* which result in unionid mortality by ammonia toxicity (Cherry et al., 2005); and bioturbation activities and organic matter enrichment due to the production of feces and pseudofeces by *C. fluminea*, which may alter sediment biochemistry and impair the survival of unionids. However, all these mechanisms remain highly speculative and to date have not been properly assessed (Sousa et al., 2008b). Furthermore, *C. fluminea* has a typical opportunistic life cycle with biological traits comprising fast growing, short lifespan, early maturation, and high fecundity, among others (Sousa et al., 2008b). By contrast, unionid mussels are usually slow-growing species, with a long lifespan and complex life history dependent of host fishes (Lopes-Lima et al., 2014). In addition to these biological traits, the invasive success of *C. fluminea* relies also on its higher tolerance to abiotic factors such as pH, salinity, and emersion than native species (Johnson & McMahon, 1998; McMahon, 2000, 2002; Sousa et al., 2008b; Ferreira-Rodríguez & Pardo, 2014, 2016).

The freshwater mussel *Unio delphinus* Spengler, 1783 is an Iberian endemism from western basins such as Miño, Douro, Tajo, and Guadiana, among others (Pérez-Quintero, 2011; Reis et al., 2013; Morais et al., 2014; Froufe et al., 2016). This species is listed as near threatened by the IUCN Red List (Araujo, 2011). The abundance and spatial distribution of *U. delphinus* populations declined in the last decades, which makes them imperative to rapidly reclassify its status (Araujo et al., 2009; Araujo, 2011). The reasons for this rapid decline are not well established. Some authors have pointed out the possibility of negative effects of *C. fluminea* on *U. delphinus*, especially when both species are found in sympatry presenting the invasive species a high density (Sousa et al., 2008a, f; Novais

et al., 2016a). However, this suggestion remains speculative and, to date, no study has addressed this issue and no attempt has been made to establish the main mechanisms behind the possible negative effects of *C. fluminea* on *U. delphinus*, or other unionid species. Given this context, this study aims to determine if *C. fluminea* interacts negatively with *U. delphinus*, potentially competing for food resources and/or space. We hypothesized that *U. delphinus* will be negatively affected, which may result in lower growth, lower physiological condition, and higher locomotor activity. In addition, we hypothesized that this interaction will be density dependent. To evaluate these hypotheses, both field and laboratorial experiments were performed to assess growth, physiological condition (carbohydrate content), and activity pattern (locomotor activity) of *U. delphinus* at different *C. fluminea* density.

## Materials and methods

Inter-specific assays using the invasive species *C. fluminea* and the native species *U. delphinus* comprised two different approaches: (i) a field experiment to determine possible negative effects on growth and physiological condition of *U. delphinus* under different *C. fluminea* density and (ii) a laboratory experiment to assess possible alteration on the activity pattern of *U. delphinus* under different *C. fluminea* density.

### Animal collection

Specimens of *C. fluminea* (5220 individuals, average length ( $\pm$ SD) of  $21.8 \pm 0.7$  mm) and *U. delphinus* (104 individuals, average length ( $\pm$ SD) of  $57.4 \pm 2.8$  mm) were collected with permission from the competent authority (Servizo de Conservación da Natureza, Xunta de Galicia) in the River Miño at 1 m depth with a kick net (1 mm mesh size).

### Field experiment

The field study was conducted in the River Miño (NW of the Iberian Peninsula). The river basin covers approximately 17,582 km<sup>2</sup>. The study area selected for the field experiment was located around 40 km upstream from the river mouth in the left bank ( $42^{\circ}04'36.81''$ N,  $8^{\circ}31'00.25''$ W, datum WGS 84), in

a slow flowing site where both *C. fluminea* and *U. delphinus* species co-exist in sympatry. The experiment was carried out in a very shallow area (total area of 20 m<sup>2</sup>, 3 m away from the bank at 1 m depth during summer, having a 100% canopy cover) with permanent freshwater conditions. In this area, the river has an annual average discharge of 326 m<sup>3</sup> s<sup>-1</sup>, with summer minimum values around 47 m<sup>3</sup> s<sup>-1</sup> and spring maximum values around 1350 m<sup>3</sup> s<sup>-1</sup> (SAICA Water Quality Network Station N015; Miño-Sil River Basin Authority). Great variations in discharge may occur on a daily basis due to the operation of a dam located 30 km upstream of the study site.

After collection, mussels were numbered using DYMO labels, and their length was measured with a digital caliper (precision of 0.001 mm). Individual *U. delphinus* were placed in a plastic box (400 × 300 × 100 mm) covered with plastic net (mesh size of 10 mm) and filled with sandy substrate (average percentage of grain size categories  $\pm$ SD: grain size <0.25 mm:  $18.942 \pm 0.816\%$ ; grain size 0.25–0.5 mm:  $43.256 \pm 3.226\%$ ; grain size 0.5–1 mm:  $37.691 \pm 2.377\%$ ; grain size >1 mm:  $0.111 \pm 0.032\%$ ; and organic matter content:  $0.342 \pm 0.283\%$ ). To examine inter-specific effects on *U. delphinus* at different *C. fluminea* density, we used four *C. fluminea* treatments of 0 (control), 100, 1000, and 2000 ind. m<sup>-2</sup> (0, 12, 120, and 240 *C. fluminea* individuals per box, respectively), using a similar size class (and biomass) between treatments in order to avoid possible bias due to size. Each treatment was replicated 10 times and boxes were randomly distributed in the river. The experiment lasted more than 5 months (between May 13 and October 31, 2015). At the end of the experiment, *U. delphinus* specimens were removed and transported to the laboratory. There, *U. delphinus* specimens were re-measured with a digital caliper and growth was calculated based on the difference between the initial and final measurements. The following abiotic factors in the water column were monthly measured from May to October 2015: temperature, conductivity, total dissolved solids, redox potential, salinity, dissolved oxygen, pH, nitrites, nitrates, ammonia, and phosphates. Information for the first seven abiotic factors was obtained in situ near the bottom, using a multiparametrical probe YSI EXO2. Nitrites, nitrates, ammonia, and phosphates were determined by colorimetric methods using the Palintest 270 standard photometer 7000 following the methodology described in Sousa et al. (2008c).

**Table 1** Summary of the criteria used for scoring the activity of *Unio delphinus* (modified from Xie & Burnell, 1995)

Score	Criteria
1	Shell valves closed
2	Shell valves opened and siphoning
3	Shell valves opened and foot clearly visible
4	Shell valves opened and foot extended out half of its full length
5	Shell valves opened and foot extended out in its full length

The physiological status of each *U. delphinus* individual was determined as carbohydrate content, which is commonly used as a biomarker to assess energy storage in freshwater bivalves (Sousa et al., 2011a; Pilotto et al., 2016). To assess the carbohydrate content, the soft tissue (whole body) of mussels was dried at 60°C for 48 h in an oven and then ground to fine powder. The carbohydrate content was measured colorimetrically using the phenol–sulphuric acid method described in Baker & Hornbach (2000).

#### Laboratory experiment

The laboratory study was conducted in September 2014 in the Marine Science Station of Toralla (ECIMAT, University of Vigo, Vigo, Spain). After collection (using the same methodology as described above), 64 *U. delphinus* (average length ( $\pm$ SD) of  $56.4 \pm 7.0$  mm) and 1500 *C. fluminea* individuals (average length ( $\pm$ SD) of  $25.0 \pm 1.0$  mm) were transported in aerated buckets with water to the ECIMAT. In the laboratory, mussels were numbered (DYMO labels), and their length was measured with a digital caliper. Mussels and clams were kept separately in 30 l aquaria for one week, with constant aeration and daily supply of freshwater microalgae *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J. Kristiansen and O. M. Skulberg (10,000 cells ml<sup>-1</sup>), in order to acclimate to laboratory conditions (20°C and 12:12 h light:darkness cycle). After acclimatization, experiments on mussel activity were conducted by placing two mussels in 30 l aquaria without the substrate daily fed with *R. subcapitata* (10,000 cells ml<sup>-1</sup>) for four days. To examine inter-specific effects on mussel activity at different *C. fluminea* densities that naturally occur in the wild (Ferreira-Rodríguez & Pardo, 2014), we used eight *C. fluminea* treatments (0—control, 25, 50, 75, 150, 300, 600, and 1200 ind. m<sup>-2</sup>). All treatments

were replicated four times, using a single *C. fluminea* size class in order to avoid possible size bias.

Under experimental conditions, *U. delphinus* were mostly active during the day (data not shown). Hence, the activity of *U. delphinus* ( $n = 64$ ) was always monitored in the morning (07:00–08:00 h, GMT), for 4 consecutive days. Mussels' activity for each treatment was measured as the average activity score of four consecutive observations using a criteria modified from Xie & Burnell (1995), as shown in Table 1. To allow animals to behave normally, mussels were observed once daily for siphoning and foot extension, without being disturbed (before daily feeding, cleaning, water exchange, and abiotic data collection). At the end of the experiment all native mussels were returned to the wild.

#### Data analysis

Growth and carbohydrate concentration data distributions were successfully tested for normality and homogeneity of variance using Kolmogorov–Smirnov Z and Levene's tests, respectively. The *C. fluminea* density effect was analyzed using univariate General Linear Model (GLM). Growth and carbohydrate content were the dependent variables and *C. fluminea* density was the fixed factor, using size as covariable for growth analysis. Growth and carbohydrate extreme outliers were identified as values lying above  $UQ + 3 \times IQR$  (Box plots; Zhang et al., 2009) and excluded from overall analysis. Differences in growth and carbohydrate concentration between treatments were tested with the Tukey HSD method. In the laboratory experiment, average activity score was graphically represented, two densities (low *C. fluminea* density:  $\leq 300$  ind. m<sup>-2</sup>; high *C. fluminea* density:  $> 300$  ind. m<sup>-2</sup>) were established and the activity score was compared using univariate general linear model (GLM). Activity score was the dependent

**Table 2** Average, minimum, maximum, and standard error (SE) values of the abiotic factors measured from May to October 2015 in the River Miño study site

	Average	Minimum	Maximum	±SE
Temperature (°C)	19.69	16.35	22.26	1.09
Conductivity ( $\mu\text{S cm}^{-1}$ )	98.83	87.00	110.00	3.96
Total dissolved solids ( $\text{mg l}^{-1}$ )	0.063	0.50	0.70	0.00
Redox potential (mV)	60.72	53.10	70.00	2.93
Salinity (psu)	0.05	0.04	0.05	0.00
Dissolved oxygen ( $\text{mg l}^{-1}$ )	9.99	9.01	10.71	0.28
pH	7.35	7.21	7.51	0.05
Nitrites ( $\text{mg l}^{-1}$ )	0.04	0.01	0.08	0.01
Nitrates ( $\text{mg l}^{-1}$ )	1.24	0.72	1.56	0.13
Ammonia ( $\text{mg l}^{-1}$ )	0.06	0.00	0.10	0.01
Phosphates ( $\text{mg l}^{-1}$ )	0.09	0.05	0.12	0.01
Hardness ( $\text{mg l}^{-1}$ )	15.83	10.00	25.00	2.01

variable and density was the fixed factor. All analyses were performed with SPSS v.18 using a significance level of 0.05.

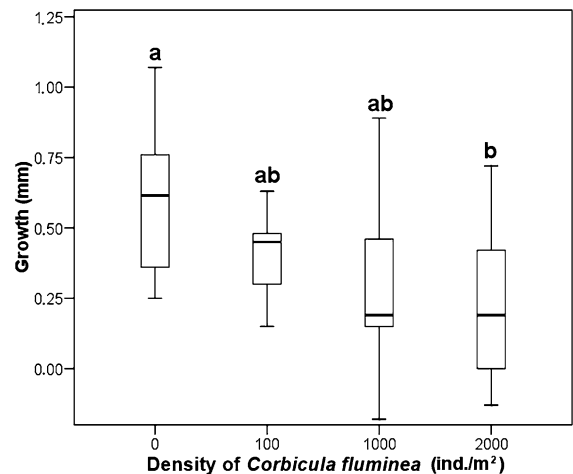
## Results

Abiotic factor values are shown in Table 2 and represent typical spring and summer conditions for the River Miño.

No mortality in *U. delphinus* (or *C. fluminea*) was recorded in any treatment during the field experiment. *U. delphinus* individuals increased in length during the experiment (Fig. 1). The GLM analysis provides evidence for an effect of *C. fluminea* density on growth. In fact, growth was significantly higher in the control treatment (no *C. fluminea*) compared to individuals growing in sympatry with *C. fluminea* ( $F_{3,40} = 3.674$ ,  $P = 0.021$ ). Average ( $\pm$ SE) growth was greater in the control treatment ( $0.599 \pm 0.098$  mm) when compared to 100 ind.  $\text{m}^{-2}$  ( $0.407 \pm 0.056$  mm), 1000 ind.  $\text{m}^{-2}$  ( $0.276 \pm 0.102$  mm), and 2000 ind.  $\text{m}^{-2}$  ( $0.233 \pm 0.101$  mm) *C. fluminea* treatments.

*Corbicula fluminea* density affected significantly the carbohydrate content of *U. delphinus* ( $F_{3,35} = 3.822$ ,  $P = 0.019$ ; Fig. 2). Average ( $\pm$ SE) carbohydrate content was  $14.078 \pm 0.357$   $\text{mg g}^{-1}$  in the control treatment and  $12.775 \pm 0.600$   $\text{mg g}^{-1}$ ,  $11.562 \pm 0.838$   $\text{mg g}^{-1}$ , and  $9.930 \pm 0.331$   $\text{mg g}^{-1}$  in 100, 1000, and 2000 ind.  $\text{m}^{-2}$  *C. fluminea* treatments, respectively.

The average activity score of *U. delphinus* was higher ( $F_{7,384} = 11.343$ ,  $P = 0.001$ ) in mussels



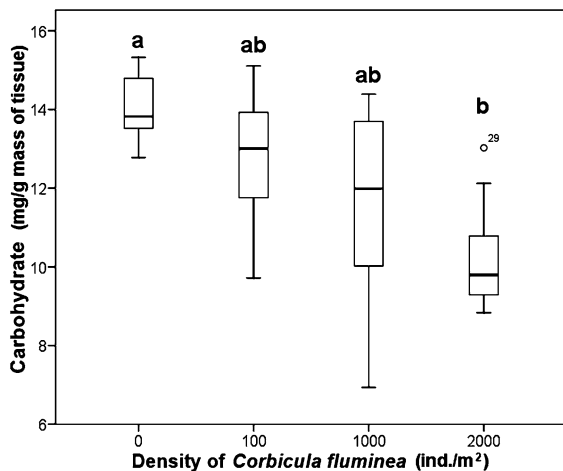
**Fig. 1** Growth of *Unio delphinus* at four *C. fluminea* densities. Box plots represent growth in ten *U. delphinus* at the end of the experiment. Each box shows the lower and upper quartile values, and the black line indicates the median value. The bars indicate the standard error, and extreme outliers identified as values lying above  $\text{UQ} + 3 \times \text{IQR}$  are omitted. Averages with a common letter do not differ from each other based on Tukey's HSD pairwise comparisons at the  $P < 0.05$  level of significance

exposed to high ( $>300$  ind.  $\text{m}^{-2}$ , Average activity score  $\pm$ SE =  $2.57 \pm 0.891$ ; Fig. 3) compared to low *C. fluminea* density ( $\leq 300$  ind.  $\text{m}^{-2}$ , Average activity score  $\pm$ SE =  $2.29 \pm 0.65$ ; Fig. 3).

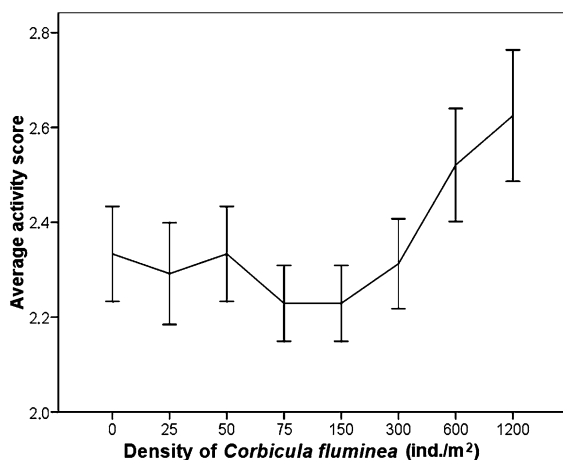
## Discussion

In the present study we demonstrated that under experimental conditions, the invasive clam *C.*





**Fig. 2** Carbohydrate content of *Unio delphinus* at four *C. fluminea* densities. Box plots represent carbohydrate content in ten *U. delphinus* at the end of the experiment. Each box shows the lower and upper quartile values, and the black line indicates the median value. The bars indicate the standard error and circles extreme values (middle outliers). Extreme outliers identified as values lying above  $UQ + 3 \times IQR$  are omitted. Averages with a common letter do not differ from each other based on Tukey's HSD pairwise comparisons at the  $P < 0.05$  level of significance



**Fig. 3** Average activity scores in *Unio delphinus* exposed to different *Corbicula fluminea* density levels. Error bars represent standard error

*fluminea* influences negatively the performance of the native freshwater mussel *U. delphinus*. Although some earlier studies (e.g., Kraemer, 1979) have shown some evidence of native unionid species declining in response to *C. fluminea* invasion in disturbed habitats, little is known about the impact of this invasive clam over native mussel populations. Thus, this study is one

of the first to assess the negative impact of this invasive clam on native freshwater mussels. In one hand, our field experiment showed that increasing *C. fluminea* densities resulted in significant slower growth and physiological condition in *U. delphinus*. On the other hand, our laboratory experiment showed significant increase in *U. delphinus* activity at high *C. fluminea* density, which may suggest an increased energy expenditure. In addition, this increased activity can be interpreted as an attempt of *U. delphinus* to avoid interaction with *C. fluminea*. Thus, it seems likely that high *C. fluminea* density may result in the displacement of native mussel species.

Results from our field experiment showed that *U. delphinus* growth was affected by *C. fluminea*. Most adult freshwater mussel species are slow-growing bivalves and typically just grow a few millimeters on a yearly basis (Neves & Moyer, 1988; Haag & Rypel, 2010). In contrast, *C. fluminea* usually presents much higher growth than unionids (maximum growth of *C. fluminea* in River Miño around 20 mm year<sup>-1</sup> in the first year of the life cycle; Sousa et al., 2008e), due to its higher filtration and assimilation rates and higher feeding plasticity (in addition to filter feeding, *C. fluminea* may also feed on organic matter deposits available in the sediments; McMahon, 2002). This higher feeding efficiency may represent a short-term advantage, mainly in oligotrophic environments. Therefore, when resources are limited in the water column, as in our study area (Dias et al., 2014), invasive fast-growing bivalves may negatively affect the survival and growth of native bivalves and may have a competitive advantage due to their higher trophic plasticity (Novais et al., 2016a). Similar results have been reported by Crooks (2001) in estuarine ecosystems using native and invasive bivalves.

*Corbicula fluminea* may also influence indirectly the unionids' physiological condition. Our results showed a decline in carbohydrate concentration as response to higher *C. fluminea* density, possibly by forcing *U. delphinus* to rely on stored reserves under these increasing *C. fluminea* densities representing stressful conditions (Gabbott & Bayne, 1973; Newell, 1985). These reserves are accumulated as carbohydrate in freshwater bivalves and are used for key metabolic and physiological processes. Therefore, carbohydrate concentrations serve as a biomarker of health condition and may reflect stress conditions in freshwater mussels, such as those resulting from *C.*

*fluminea* invasion (Lagadic et al., 1997; Hamza-Chaffai et al., 2003).

Results from our laboratory experiment showed an increase in activity of *U. delphinus* when subjected to high *C. fluminea* densities. The ecological requirements of both species can sometimes overlap; habitats with muddy and sandy sediments rich in organic matter are easily colonized by unionids as well as *C. fluminea* (Vaughn & Spooner, 2006; Sousa et al., 2007; Ferreira-Rodríguez & Pardo, 2014). Indeed, in the study area where the field experiment was conducted (River Miño) both species live in sympatry (Sousa et al., 2005). Given our laboratory results, *C. fluminea* density above 300 ind. m<sup>-2</sup> may represent stressful conditions for *U. delphinus* individuals, displacing them to lower quality habitats. However, it must be taken into account that predictions made under experimental conditions may fail in nature. For example, Englund & Heino (1994) have demonstrated increases in daily activity rhythm in caged mussels. Hence, since caged mussels are unable to burrow to great depths, and activity patterns depend on a wide range of abiotic (e.g., temperature, light, endogenous rhythms, or algal concentrations; McCorkle et al. 1979; Amyot & Downing, 1997; Riisgård et al., 2003) and biotic (e.g., parasitism, predation) factors, more studies under natural conditions are necessary to better understand the relationship of the observed activity pattern in *C. fluminea* density.

Although we demonstrate the negative effects of *C. fluminea* over *U. delphinus*, our experimental approaches were not able to determine the main mechanisms behind this interaction. However, inter-specific competition for space and/or resources and abiotic changes resulting from high *C. fluminea* density may be involved in the negative impacts they cause on native unionids. By definition, competition is a negative interaction between two or more species of the same trophic level, which exploit the same shared, limiting resources (Connell, 1983). Our results suggest that *U. delphinus* was negatively affected and that this may be due to *C. fluminea*'s superior exploitative competitive ability, as described above. It seems reasonable to expect that there could be some reciprocity (Connell, 1983), with *U. delphinus* negatively affecting *C. fluminea*. However, a different experimental approach will be necessary to prove this situation.

The possible resulting biological competitive interaction is often assumed to be a key aspect influencing

invasion success and many examples in the literature show how invasive species can outcompete native species in terms of space (Carlton et al., 1999; McIntosh et al., 1992) and food resources (Byers, 2000; Alcaraz et al., 2008), mainly when those are scarce and become insufficient to support all organisms (Wiens, 1977; Gurnell et al., 2005). First, food resources are a limiting factor in any animal species. Recent experimental works with mollusks, including gastropods (Byers, 2000) and bivalves (Byers, 2005; Crooks, 2005), have demonstrated that in a competition context the resource levels may experience differences in growth. However, the real advantage of one species depends on its ability to effectively convert resources into energy (Murdoch et al., 1996; Byers, 2000). A reduction in energy uptake due to resource competition with *C. fluminea* is a very likely explanation for the reduced growth in *U. delphinus*. Several examples in the literature exist relating these differences in growth with inter-specific competition (e.g., Bayne et al., 1979; Connell, 1983; Belanger, 1991; Cope & Winterbourn, 2004). Other similar studies, performed in the field and laboratory in USA and UK, have shown that zebra mussels also reduced the energy stored in freshwater mussels due to food resources competition (Baker & Hornbach, 2000; Sousa et al., 2011a). This situation resulted in decreases of native unionids' physiological condition, compromising their reproduction and recruitment rates in areas with high density of invasive bivalves (Tlili et al., 2011; Tankoua et al., 2012). Second, space is also a limiting factor for benthic invertebrates and some studies have shown that the potential bioturbation by *C. fluminea* could influence benthic characteristics, displacing and/or reducing available habitats for native species, mainly at high density (Hakenkamp et al., 2001; Vaughn & Hakenkamp, 2001). Besides displacement to lower quality habitats, competition has been linked to risky behavioral alterations (e.g., mortality due to high flow rates or predation; Block et al., 2013) and reductions in reproductive success because mussels depend on spatial aggregations to maximize fertilization success (Downing et al., 1993). In addition, behavioral impairments increase energy expenditure and represent an additional energetic cost (Schoener, 1983; Trueman, 1983) that could compromise the expensive gamete production (especially in females). Until now, many locomotor activities were previously described in freshwater mussels in

association with mussel community structure (Allen & Vaughn, 2009), seasonal changes in abiotic factors (Amyot & Downing, 1997), reproduction (Amyot & Downing, 1998), or predation (Jokela & Mutikainen, 1995), but never in association with invasive species. Finally, *C. fluminea* can be responsible for changes in abiotic conditions, which may interfere negatively with native unionids. For example, bioturbation, production of faeces and pseudofaeces, or *C. fluminea* massive mortalities can change sediment characteristics, organic matter content, oxygen, and nutrient cycling impairing the survival of freshwater mussels (Sousa et al., 2014). Unfortunately, many of these abiotic changes and their direct negative effects on freshwater mussels remain untested and further studies are necessary to clarify these effects.

Our results could explain, in part, the loss of up to 30% of *U. delphinus* populations in the Iberian Peninsula in the last decades (Araujo, 2011). However, the introduction of new competitors rarely causes native species extinctions, with most existing studies only able to show declines in abundance but not extinction or extirpation (Bruno et al., 2005). In addition, the introduction of invasive species are not the unique threat affecting Iberian Peninsula unionid populations, as many ecosystems are currently subjected to habitat loss, fragmentation and degradation due to anthropogenic activities, changes in the hydrologic regime due to dams and impoundments, water abstraction, loss of host fish populations, and water pollution (Araujo et al., 2009; Araujo, 2011; Douda et al., 2013). Therefore, the introduction of invasive species such as *C. fluminea* to ecosystems where native mussels are already in decline is an additional threat that should not be neglected.

## Conclusion

The biological traits (e.g., long life cycle, slow growth, parasitic larval stage) of freshwater mussels render them particularly susceptible to abiotic and biotic disturbances (Lopes-Lima et al., 2014, 2016). In this study, we have shown that *C. fluminea* invasion may negatively affect the native freshwater mussel *U. delphinus* in Iberian Peninsula, whose populations are already declining. Our findings suggest that *C. fluminea* can negatively affect growth, physiological condition, and locomotor activity of native unionids,

and this interaction is particularly detrimental to *U. delphinus* at sites with high *C. fluminea* density. Therefore, future studies should aim to evaluate the impact of *C. fluminea* on other threatened mussel species, evaluate similar effects on unionid juveniles (possibly even more vulnerable than adults), and assess possible spatial (sites subjected to different abiotic conditions and food resource levels) and temporal (winter versus summer conditions) variations. Given the widespread distribution of *C. fluminea* and the declining conservation status of many freshwater mussel species worldwide, the effects of Asian clams should be taken into account in future management actions devoted to the conservation of native unionids.

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