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Potential impacts of the invasive species *Corbicula fluminea* on the survival of glochidia

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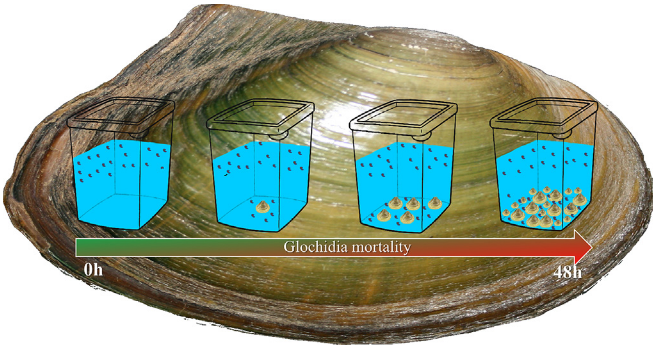
HIGHLIGHTS

- *C. fluminea* influence on the mortality of native mussels glochidia was evaluated.
- Glochidia mortality was highly dependent on *C. fluminea* density and time.
- Glochidia mortality was influenced by *C. fluminea* activities.
- High *C. fluminea* densities may be detrimental for freshwater mussel conservation.

GRAPHICAL ABSTRACT



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ABSTRACT

Freshwater mussels (Bivalvia, Unionida) are one of the most imperilled faunal groups globally, being the introduction of invasive species a possible major mechanism of threat. The Asian clam *Corbicula fluminea* is a problematic invasive species in aquatic ecosystems and can impair the survival of parasitic larvae (glochidia) of native freshwater mussels. However, this possible mechanism of threat remains speculative and to date very few studies addressed quantitatively this issue. In order to cover this gap, we have performed a series of manipulative laboratory studies to assess how distinct densities of *C. fluminea* can affect the survival of glochidia after 6, 12, 24 and 48 h of exposure, using larvae of the native freshwater mussel *Anodonta anatina*. Our results suggest an increase in mortality of *A. anatina* glochidia with an increase in density of *C. fluminea*. Two main mechanisms may possibly explain our results: 1) the high filtration capacity of *C. fluminea* that can contribute to the mortality of glochidia due to the mechanical damage of their fragile shells when passing by siphons and/or digestive tract of *C. fluminea* and 2) the high excretion capacity of *C. fluminea* that can lead to mortality of glochidia due to increase in ammonia concentration. Mortality of glochidia was also time dependent with higher values registered after 48 h. This work is one of the first showing the influence of *C. fluminea* density on the survival of glochidia, being filtration (and consequent passage in the digestive tract) and biodeposition the main potential mechanisms explaining overall mortality. These results also suggest that sites with high densities of *C. fluminea* may be highly detrimental for the conservation of freshwater mussels, potentially impairing the survival of glochidia and negatively affecting the recruitment of juveniles.

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1. Introduction

Over the last decades the interest about species with a complex life-cycle has been increasing among biologists (Allen et al., 2018; Combes, 1997; Rauch et al., 2005; Roughgarden et al., 1988; Stoks and Córdoba-Aguilar, 2012; Wilbur, 1980). Usually, species with complex life-cycle encompass organisms that undergo metamorphosis, together with its associated changes on morphology, physiology and behaviour, at least once in their life history (Hadfield, 2000; Hyeun-Ji and Johansson, 2015; Rowe and Ludwig, 1991; Werner, 1988). Of the >80% of animals that undergo metamorphosis (Werner, 1988; Wilson and Krause, 2012), many are parasitic at least in some life stages (Galetti et al., 2018; Stoks and Córdoba-Aguilar, 2012; Wilbur, 1980).

Contrary to terrestrial invertebrates, where larvae are generally specialized on growth and adults on dispersion, parasitic aquatic invertebrates may have a high dispersal capacity during their larval stage, whereas adults may be sedentary (Ueda et al., 2016; Wilbur, 1980). An interesting example of aquatic organisms that have a complex life-cycle comprises freshwater mussels (Bivalvia, Unionida) (Modesto et al., 2018). They are a diverse animal group distributed worldwide except Antarctica, being highly threatened and suffering major declines over the last century (Lopes-Lima et al., 2014, 2018). The life cycle of freshwater mussels includes a parasitic life stage as larvae (named glochidia), which are released by females and need to attach to fish hosts (rarely attaching to other vertebrates) to continue its development into juvenile mussels (Barnhart et al., 2008; Douda et al., 2013; Lopes-Lima et al., 2016; Modesto et al., 2018). The poor conservation status of freshwater mussels is associated, in part, to their complex and unique life-cycle in addition to the increased of human disturbance on freshwater ecosystems (Lopes-Lima et al., 2017; Santos et al., 2015; Sousa et al., 2014; Strayer et al., 2004).

Probably one of the major drivers of native freshwater mussels' declines is the introduction of invasive alien species (IAS) (Bódis et al., 2014a; Ferreira-Rodríguez et al., 2018; Sousa et al., 2011a, 2014). IAS are considered a serious threat to native freshwater mussels due to direct competition for food and space, introduction of diseases and parasites and being also responsible for abiotic changes that can disrupt the dynamics of native populations (Boltovskoy and Correa, 2014; Sousa et al., 2005, 2014; Strayer et al., 2004). Bivalves, including species such as *Corbicula fluminea* (Müller, 1774), *Dreissena polymorpha* (Pallas, 1771), *Limnoperna fortunei* (Dunker, 1857), *Sinanodonta woodiana* (I. Lea, 1834), among others, are considered problematic in freshwater ecosystems, since they can occur at remarkably high densities (Balogh et al., 2008; Bódis et al., 2011, 2014b, 2016; Karatayev et al., 2010; Sousa et al., 2008a, 2008d, 2014).

A relevant example is the invasive bivalve *Corbicula fluminea* (Müller, 1774) that due to its activities (i.e. burrowing and bioturbation, suspension and deposit feeding, high filtration rates; Ilarri and Sousa, 2012), high density and biomass, and opportunistic behaviour (i.e. ability to colonize different environments, high phenotypic plasticity, high tolerance to abiotic fluctuations, shorter-life cycle, rapid growth, earlier sexual maturity, and great fecundity; Ilarri and Sousa, 2012; McMahon, 2002; Sousa et al., 2008a) have successfully colonized new environments and negatively affected native species (Sousa et al., 2008a). On the invaded ecosystems, high densities of *C. fluminea* can be responsible for impacts ranging from the individuals to the ecosystem (Ilarri et al., 2012, 2015; Ilarri and Sousa, 2012; McMahon, 2000; Novais et al., 2016; Phelps, 1994; Sousa et al., 2009, 2014; Zhang et al., 2011). *Corbicula fluminea* can change the invaded area through its activities which may also impact native freshwater mussel populations (Haag et al., 1993; Ricciardi et al., 1997; Sousa et al., 2009, 2014; Strayer, 1999). Although there are several studies focusing on *C. fluminea* impacts, only a few have evaluated their negative effects on native freshwater mussels (see Ferreira-Rodríguez et al., 2018; Cherry et al., 2005; Cooper et al., 2005). However, must of these negative impacts on native freshwater mussels remain speculative and untested.

Due to this lack of knowledge, it is crucial to study the possible impacts of *C. fluminea* on freshwater mussels in more detail, especially those that can possibly affect glochidia and the recruitment of juveniles, since this can be the most vulnerable phase of their life cycle (Ferreira-Rodríguez et al., 2019). Therefore, the main goal of the present study is to evaluate, through manipulative experiments, the influence of the invasive bivalve *C. fluminea* on the glochidia survival of the native freshwater mussel species *Anodonta anatina* (Linnaeus, 1758). The freshwater mussel *A. anatina* has a wide distribution that includes Europe, Asia and North Africa, being considered a generalist species regarding habitat preferences (Froufe et al., 2014; Hinzmann et al., 2013; Lopes-Lima et al., 2017). Given the usual overlap in distribution between *A. anatina* and *C. fluminea* in Europe, the present study aimed at evaluating: (1) the effect of *C. fluminea* activities (filtration and biodeposition) and (2) the effect of different ammonia concentrations (simulating *C. fluminea* faeces and pseudofaeces production) on the survival of *A. anatina* glochidia. We hypothesized that the increase of filtration and ammonia concentration will negatively affect the survival of *A. anatina* glochidia and this situation will be density and time dependent.

2. Methods

2.1. Organisms collection

Corbicula fluminea (130 individuals, average ± SD size of 30 ± 10 mm) specimens were collected in Praia da Lenta (41°57′N, 08°44′W), a semi-enclosed area in the Minho River located 14 km upstream the mouth of river, on January 2018 (for a description of the collection site see Sousa et al., 2013). Minho River is a low human-impacted system, although in the last decades has been subjected to several IAS introductions (for a revision see Sousa et al., 2008c). In fact, the invasive species *C. fluminea* was first described for the Minho River in 1989 (Araujo et al., 1993) and currently it contributes with >90% of the benthic estuarine biomass (Sousa et al., 2008f).

Anodonta anatina (6 individuals, average ± SD size of 97 ± 13 mm) specimens were collected in Tua River, upstream of the city of Mirandela (41°30′N; 7°11′W), on January 2018 (the end of the reproduction season of the species in the North of Portugal; Hinzmann et al., 2013). Tua River basin is a well-preserved area in which several native freshwater mussel species (*A. anatina*, *Margaritifera margaritifera* (Linnaeus, 1758), *Potomida littoralis* (Cuvier, 1798), *Unio delphinus* (Spengler, 1793)) occur (Sousa et al., 2012). The invasive bivalve *C. fluminea* also occurs in the Tua basin (Sousa et al., 2012).

2.2. Manipulative experiments

Invasive bivalves and native freshwater mussels were collected and immediately transported to CIIMAR (Interdisciplinary Centre of Marine and Environmental Research, University of Porto) laboratorial facilities. During the two weeks before the beginning of the experiment, organisms were kept in two aquariums with a total volume of 35 L with flowing, dechlorinated, and aerated distilled water (acclimation period). The photoperiod (12:12 h of a light-dark cycle) and temperature (15 °C) were kept constant. All organisms were fed daily with *Chlorella vulgaris*.

In the first laboratorial experiment we evaluated the influence of different *C. fluminea* densities on the survival of *A. anatina* glochidia. In the second laboratorial experiment we evaluated the influence of different ammonia concentrations on the survival of *A. anatina* glochidia. The two manipulative experiments were performed in microcosms with 10 × 10 × 18 cm (100 cm^2 of area), filled with 500 mL of distilled water.

2.2.1. Influence of Corbicula fluminea on the survival of Anodonta anatina glochidia

In this manipulative experiment four treatments (n = 5 per treatment) were used: (1) control (without *C. fluminea*), (2) 1 ind. 100 cm^{-2}

(low density of *C. fluminea*), (3) 5 ind. 100 cm^{-2} (intermediary density of *C. fluminea*) and (4) 20 ind. 100 cm^{-2} (high density of *C. fluminea*). This range of *C. fluminea* densities were selected based on values present on Minho River, that usually range from 500 ind. m^{-2} to 2000 in. m^{-2} (Sousa et al., 2008b, 2008d).

Corbicula fluminea individuals were placed in aerated microcosms in the laboratory. Three days after the inclusion of *C. fluminea*, samples with 100 *A. anatina* glochidia were included in each microcosm using a 3 mL pipette. Glochidia were collected from gravid females by opening their valves, placing a water-filled syringe into the marsupial gill, and promoting the release of glochidia. The evaluation of glochidia viability was checked by their valve's movement when in contact with a NaCl solution. Samples of one hundred glochidia were separated to different 20 mL recipients and then transferred to the microcosms. Thereafter, samples were collected at different time periods: 6 h (T1), 12 h (T2), 24 h (T3) and 48 h (T4). Along the different time periods, each sample was filtered with a 120 μm mesh sieve. Afterward, with the use of a needle, the live and dead glochidia present in each sample were counted. Glochidia with movement were considered as being alive. On contrary, if the valves of the glochidia were broken or without movement they were counted as dead. Total ammonia concentration (considering 97.5% NH_4^+ + 2.5% NH_3) in the water in each microcosm and time period was assessed using the Auto-analyzer Colorimetric Skalar SanPlus Flow Segmented, Skalar M155-008R (EPA 350.1) method.

2.2.2. Influence of ammonia on the survival of Anodonta anatina glochidia

The same experimental design was used to evaluate the possible influence of ammonia on the survival of *A. anatina* glochidia. A total of nine treatments and eight different concentrations of total ammonia (considering 97.5% NH_4^+ + 2.5% NH_3) were tested: (1) control, (2) 0.05 $mg \cdot L^{-1}$, (3) 0.10 $mg \cdot L^{-1}$, (4) 0.25 $mg \cdot L^{-1}$, (5) 0.5 $mg \cdot L^{-1}$, (6) 1 $mg \cdot L^{-1}$, (7) 2.5 $mg \cdot L^{-1}$, (8) 5 $mg \cdot L^{-1}$, (9) 10 $mg \cdot L^{-1}$. The lowest five concentrations were selected to mimic the concentrations of ammonia measured during the first experiment (see above). The other four concentrations were selected taking into account the amount of ammonia in water that is considered to be toxic in Portuguese ecosystems (Ministério do Ambiente, 1998). During the experiment pH and temperature were measured using a multi-parameter analyzer Consort C562 since ammonia concentrations may be affected by these abiotic factors (see Wang et al., 2008). As in the previous experiment (see above), live and dead glochidia were counted at the same time periods T1, T2, T3 and T4 as described above.

2.3. Data analysis

To evaluate the possible influence of *C. fluminea* density and ammonia concentration on survival of *A. anatina* glochidia over time (first laboratorial experiment), a Generalized Linear Model (GLM) was fitted to the data. Mortality of *A. anatina* glochidia in the different densities of *C. fluminea* and time were modelled using a negative binomial (NB) distribution (following Zuur et al., 2009), given that the preliminary analysis showed that the data had over-dispersion (i.e. the variance of the data were much higher than the average). The model included a response variable: mortality of *A. anatina* glochidia, and two predictor continuous variables: *C. fluminea* density and time. In addition, given that ammonia excretion by *C. fluminea* accumulates over time and depends on density, this variable was included into the model as a continuous covariate. The residuals of the model were checked regarding the assumptions of normal and homogeneous error, and for the goodness of fit it was assessed with the Hosmer-Lemeshow Goodness of Fit (GOF) test from the package ResourceSelection (Lele, 2009). The statistical significance of variance used were of $\alpha = 0.05$. The data was modelled with the *glm.nb* function from the package MASS (Venables and Ripley, 2002) implemented in R (R Core Team, 2014).

To assess the possible influence of ammonia on the survival of *A. anatina* glochidia over time (second laboratorial experiment), a two-way PERMANOVA (type III) was used. The mortality of *A. anatina* glochidia over time was tested with a two-way PERMANOVA (type-III), with ammonia (nine levels: [control], [0.05], [0.10], [0.25], [0.50], [1], [2.5], [5], [10]) and time (four levels: T1, T2, T3 and T4) as fixed factors. Prior to the analyses, data were fourth root transformed and a resemblance matrix based on the Euclidean distance was calculated. For the PERMANOVA tests the statistical significance of variance ($\alpha = 0.05$) was tested considering 9999 permutations of residuals within a reduced model. If the number of permutations were lower than 150, the Monte Carlo *p*-value was considered. Pairwise comparisons were performed for all PERMANOVA tests. PRIMER software (version 6.0 with the PERMANOVA + add-on package; Clarke and Gorley, 2006; Anderson et al., 2008) was used for the PERMANOVA tests.

3. Results

3.1. Influence of Corbicula fluminea on the survival of Anodonta anatina glochidia

The mortality of *A. anatina* glochidia increased with higher densities of *C. fluminea* and over time (Fig. 1). Mortality varied between 3.4 (± 1.82) in control during T1 and 53.0 (±4.06) in the treatment with 20 ind. 100 cm^{-2} during T4.

Ammonia concentration increased with higher densities of *C. fluminea* and over time (Fig. 2). Ammonia varied between 0.02 (± 0.006) $mg \cdot L^{-1}$ in control during T1 and 1.45 (±0.970) $mg \cdot L^{-1}$ in the treatment with 20 ind. 100 cm^{-2} during T3.

Mortality of *A. anatina* glochidia positively responds to the variables ammonia, density of *C. fluminea* and time, the GLM indicated a positive significant correlation of *A. anatina* glochidia mortality with these variables (Table 1). The GLM had a predictive capacity of $r^2 = 0.57$.

3.2. Influence of ammonia on the survival of Anodonta anatina glochidia

Mortality of *A. anatina* glochidia increased with higher ammonia concentrations and over time. Mortality varied between 1.0 (±1.73) in control during T1 and 66.2 (±6.38) in the treatment with 10 $mg \cdot L^{-1}$ during T4 (Fig. 3).

Mortality of *A. anatina* glochidia varied significantly in response to ammonia concentration and time (Table 2). Regarding ammonia concentrations, the pairwise tests showed that the mortality varied between the lower (from 0.05 to 0.5 $mg \cdot L^{-1}$) and higher (from 1 to 10 $mg \cdot L^{-1}$) ammonia concentrations (Fig. 3). For time, the differences on pairwise tests were between all times of the experiment with exception of T2 with T3 (Fig. 3).

Mortality of *A. anatina* glochidia was recorded for all ammonia concentrations (Fig. 3). However, a mortality of 50% of *A. anatina* glochidia

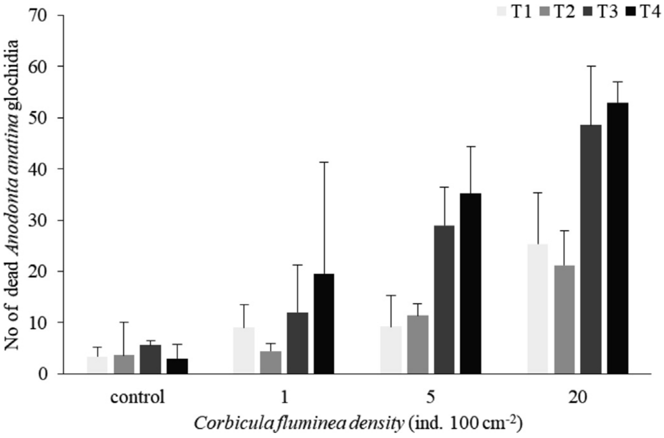

Fig. 1. Average (+SD) values of dead *Anodonta anatina* glochidia in four different densities of *Corbicula fluminea* and over time.

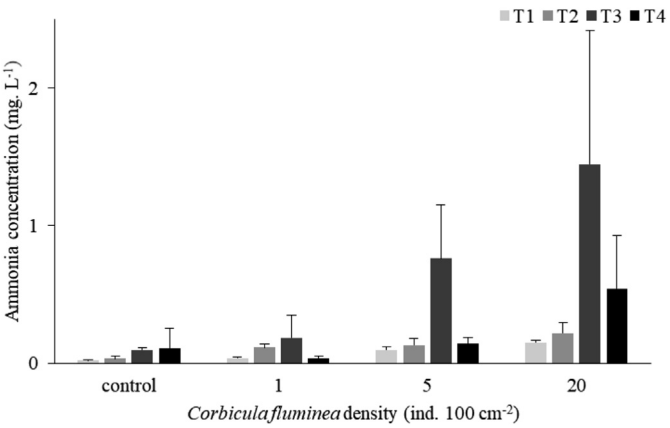

Fig. 2. Average (+SD) values of ammonia concentration in four different densities of *Corbicula fluminea* and over time.

was only observed for the ammonia concentration of 10 mg·L^{-1} and only after 48 h (T4) (Table 3).

4. Discussion

High mortality rates during the glochidia and juvenile stages, due to failures in attachment to appropriate fish hosts and settlement in unsuitable habitats, are well known in freshwater mussel species (Cherry et al., 2005; Modesto et al., 2018; Neves and Widlak, 1987). Indeed, the number of glochidia produced by a female freshwater mussel can reach >10,000,000; however, most species have very low survival rates of glochidia with 99.99% failing to attach to a suitable host and therefore not completing their development into juveniles (Bauer, 1987; Haag, 2012, 2013; Jansen et al., 2001; Modesto et al., 2018; Strayer, 2008; Young and Williams, 1984). As such, information regarding the survival of freshwater mussels during this early stage of their life-cycle is crucial to the conservation of these species (Ferreira-Rodríguez et al., 2019). The present study showed that *C. fluminea* can negatively affect the survival of *A. anatina* glochidia, with possible repercussions in their recruitment and future conservation, and shed light about the main mechanisms responsible for this mortality. Similar results (or even more critical) are expected for other freshwater mussel species (see further discussion below).

IAS can strongly affect the abundance of native mussels (Strayer, 1999). In this regard, the effects of some well-known invasive species (*Dreissena polymorpha* and *Limnoperna fortunei*) have been well documented (Carlsson et al., 2009; Clayton et al., 2001; Karatayev et al., 2007; Kat, 1986; Mansur et al., 2012; Ricciardi et al., 1998; Sousa et al., 2011b). In contrast, for the Asian clam *C. fluminea*, and despite the numerous studies suggesting their negative effects on native freshwater mussels, few included quantitative evidences of these impacts (Cherry et al., 2005; Cooper et al., 2005; Ferreira-Rodríguez et al., 2018; Neves and Cherry, 1999; Scheller, 1997). Not to mention that in most cases these studies have focused in the impacts on young juveniles (immediately following metamorphosis) or adults (Cherry et al., 2005; Cooper et al., 2005; Ferreira-Rodríguez et al., 2018; Neves and Cherry, 1999; Scheller, 1997). For example, in a recent study, Ferreira-Rodríguez et al. (2018) reported lower growth and physiological condition, and higher locomotor activity in *U. delphinus* adults in the presence of higher *C. fluminea* densities. Other studies described that massive mortalities of *C. fluminea* are also associated to the mortality of *Villosa iris* (Lea, 1829) and *Pyganodon grandis* (Say, 1829), both adults and juveniles, due to increased ammonia toxicity during the decomposition process (Cherry et al., 2005; Cooper et al., 2005). As far as we know, Neves and Cherry (1999) is the only study that have demonstrated a negative correlation between glochidia abundance (using *V. iris*) and the presence of *C. fluminea*. The authors concluded that this negative correlation was probably a result of *C. fluminea* ingestion of glochidia during filtration and consequent release of these glochidia on their faeces and/or pseudofaeces.

Table 1
Generalized linear model outputs of the model fitted to quantify the variation of dead *Anodonta anatina* glochidia throughout different densities of *Corbicula fluminea* and ammonia concentration over time (negative binomial model).

	Estimate	SE	z-value	*p* value
Intercept	1.21	0.21	5.78	*<0.05*
Ammonia	3.89	1.36	2.87	*<0.05*
Density	0.07	0.02	4.14	*<0.05*
Time	0.03	0.01	4.57	*<0.05*
Ammonia:Density	−0.09	0.07	−1.36	0.17
Ammonia:Time	−0.05	0.04	−1.21	0.22

Italics refer to significant *p* value.

The Asian clam *C. fluminea* has a high filtration capacity (it can range from 29 to 3252 ml. h^{-1}. ind^{-1}) compared to other bivalves (Boltovskoy et al., 1995; Cohen et al., 1984; Viergutz et al., 2012; Vohmann et al., 2010). Since *C. fluminea* can attain remarkable densities on the invaded ecosystems (Cataldo and Boltovskoy, 1999; Cohen et al., 1984; Modesto et al., 2013; Phelps, 1994; Sousa et al., 2008e) this filtration capacity can remove food resources or impair the reproduction and/or recruitment of native freshwater mussels. In fact, *C. fluminea* has been cited as an important threat to reproduction or recruitment of native freshwater mussels, either by removing sperm or in a latter phase being responsible for the mortality of glochidia and/or newly metamorphosed juveniles (Sousa et al., 2014; Strayer, 1999). In addition, the increase of toxic ammonia as a result of great quantities of faeces and pseudofaeces production or during massive mortalities has been also described as an important possible mechanism of threat to freshwater mussels (Scheller, 1997; Sousa et al., 2014; Strayer, 1999; Neves and Cherry, 1999).

In the present study, the activities of *C. fluminea* (filtration and biodeposition) probably contributed to the mortality of *A. anatina* glochidia. This was especially valid for the treatments with densities higher than 20 ind. 100 cm^{-2} (2000 ind. m^{-2}), and that were in contact with *C. fluminea* for >48 h. Since *C. fluminea* filter suspended particles across the gill (McMahon, 1991), when the food and non-food particles enter via the incurrent siphon, the gill works as a sieve concentrating the smaller (in the range of 15–25 μm) particles (Atkinson et al., 2011; Bayne and Newell, 1983; Decho and Luoma, 1991). Once inside the gills, particles can follow two different pathways. Food particles can move across from the anterior end of the gills to the labial palps, then the anterior food grove to the esophagus and finally to the stomach and gut (Cummings and Bogan, 2006). When these particles are big, they might be fragmented in smaller fractions due to the complex digestive system of *C. fluminea* (Decho and Luoma, 1991; McMahon, 1991). In fact, these clams have a highly efficient digestive system, which allows them to return undigested particles from the stomach to the gut several times for further assimilation (Decho and Luoma, 1991; McMahon, 1991). Finally, these particles can be excreted as faeces that are released involved on mucous secreted by the gut and rectal cells through the excurrent siphon (Decho and Luoma, 1991; McMahon, 1991; Cummings and Bogan, 2006). On the other hand, non-food particles can be sorted, moving off the ventral margin of the gills, and can be released through the incurrent siphon or mantle bounded by the mucous formed along the pedal gape as pseudofaeces (Cummings and Bogan, 2006; Decho and Luoma, 1991; McMahon, 1991). Therefore, considering that the size of *A. anatina* larvae is much higher than the particles filtered by *C. fluminea* (in Portuguese populations the size of *A. anatina* glochidia may vary between 340 and 566 μm; Lopes-Lima et al., 2016), these glochidia when filtered and ingested might be fragmented in small pieces. These glochidia are probably not assimilated by *C. fluminea*, and consequently are returned from the stomach to the gut several times being fragmented, killed and excreted as faeces, or even

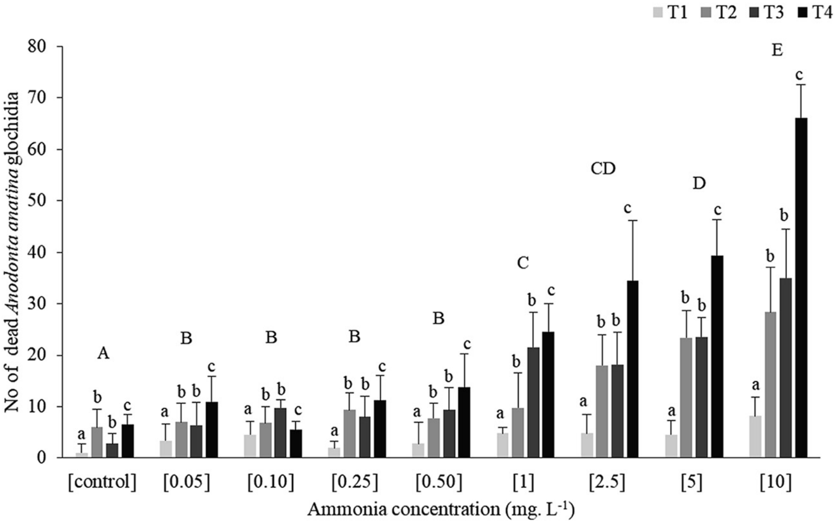

Fig. 3. Average (+SD) values of dead *Anodonta anatina* glochidia in nine different ammonia concentrations and over time. Uppercase letters refer to comparisons among ammonia concentrations, while lowercase letters to comparisons among time periods.

entangled in the mucous as pseudofaeces (Neves and Cherry, 1999). In the present study most of the dead glochidia observed in the first experiment were found in these conditions (i.e., broken in small fragments and entangled in mucous), with the higher density of *C. fluminea* and longer exposures contributing to an increasing mortality.

Although this experiment was just focused on the *A. anatina* glochidia it is possible that similar results would be obtained with other freshwater mussel species. In fact, the size of glochidia in other species are usually smaller (range between 70 and 385 μm; Araujo et al., 2009; Bauer, 2001; Kennedy and Haag, 2005) and theoretically more sensitive than described for *A. anatina* in this study. Therefore, future studies should conduct similar experiments in order to assess the impacts of *C. fluminea* on glochidia survival of other freshwater mussel species that live in sympatry with this IAS.

In the present study it was also possible to observe that faeces and pseudofaeces of *C. fluminea* do not seem to produce levels of ammonia (between 0 and 0.5 $mg \cdot L^{-1}$) high enough to strongly affect the *A. anatina* glochidia; however, some mortality was directly related to the ammonia levels. The ammonia measured in the study was the total ammonia, including the unionized (NH_3) and the ionized (NH_4^+) ammonia. Although NH_3 is very toxic to aquatic organisms (Erickson et al., 2008; Redner and Stickney, 1979), including the glochidia of freshwater mussels (Augspurger et al., 2003; Wang et al., 2007a), *A. anatina* glochidia showed a high tolerance to ammonia toxicity in the second experiment, since the EC_{50} was only reached at 10 $mg \cdot L^{-1}$ of total ammonia after 48 h (Table 3). Ingersoll et al. (2006) reported that many species belonging to the subfamily Anodontinae have highly resilient glochidia, since after being discharge by females, they remain viable for days in the water before attachment to the fish hosts. This resilience varies between different freshwater mussel subfamilies, being usually higher in the subfamily Anodontinae (Table 3). Wang et al. (2007b) described a lower toxicity resistance by *Venustaconcha ellipsiformis* of the subfamily Ambleminae, wherein the EC_{50} of glochidia tested was 2.7 $mg \cdot L^{-1}$ of total ammonia concentration after 48 h.

With the increasing distribution of the *C. fluminea* worldwide (Crespo et al., 2015), and the high densities that this species can attain, the possibility that this IAS dominate the benthic biomass and negatively affect the native freshwater mussel species is high. The results reported here strongly suggest that the invasive bivalve *C. fluminea* can negatively influence the recruitment of the native freshwater mussel *A. anatina* causing the mortality of their glochidia. Thus, invasion of *C. fluminea* is a plausible, but possibly overlooked, explanation for some of the declines in freshwater mussels registered in the last decades (Haag, 2012). Evidences that *C. fluminea* has strong and pervasive negative effects in freshwater mussels is still debatable. In fact, some authors described that *C. fluminea* and the native freshwater mussels occupy different microhabitats, with *C. fluminea* occurring in high densities in areas that the freshwater mussels occur in low densities (Vaughn and Spooner, 2006). This non-overlapping distribution may indicate (among other hypotheses) that freshwater mussels are not able to persist in high density areas of *C. fluminea*. In addition to possible competition for food and habitat, our study showed that *C. fluminea* can increase the mortality of glochidia, and this could lead to possible negative effects on the recruitment of freshwater mussels.

Table 2
Summary of two-way PERMANOVA results comparing the number of dead *Anodonta anatina* glochidia in different ammonia concentrations and over time.

Source	df	SS	MS	Pseudo-F	P
Ammonia	8	20.34	2.54	26.49	*<0.05*
Time	3	18.19	6.06	63.18	*<0.05*
Ammonia × Time	24	3.62	0.15	1.57	0.05

Italics refer to significant *p* value.

5. Conclusions

Corbicula fluminea is recognized as one of the most problematic IAS in freshwater ecosystems and is usually described as a possible threat to the conservation of native freshwater mussels. Despite of this, to date very few studies were able to provide quantitative evidences of this situation. Results of the present study showed that the mortality of the native freshwater *A. anatina* glochidia increased with higher densities of *C. fluminea* and when submitted to long time periods in contact with this IAS. Filtration and consequent ingestion of glochidia and biodeposition activities were the main possible mechanisms responsible for the high mortalities registered. It was also possible to observe that *C. fluminea* do not seem to produce levels of ammonia high enough to strongly affect *A. anatina* glochidia. Though, more manipulative experiments are necessary to better understand and to compare the effects of the invasive *C. fluminea* in other freshwater mussel species that are highly threatened and live in sympatry with this IAS.

Table 3
EC_{50} (effective concentration responsible for the survival of 50% of *Anodonta anatina* glochidia in ammonia toxicity tests) comparison of this and other studies made with freshwater mussel species during a 48 h period.

Species	Subfamilies	Total ammonia EC_{50} (mg $N \cdot L^{-1}$)	References
		48 h	
Anodonta anatina	Anodontinae	10.0	Present study
Actinonaias ligamentina	Ambleminae	3.3–8.0	
Lampsilis siliquoidea	Ambleminae	7.7–13.0	Wang et al., 2007a
Actinonaias ligamentina	Ambleminae	3.3–8.0	
Alasmidonta heterodon	Anodontinae	15.0–16.0	
Lampsilis fasciola	Ambleminae	4.0–6.7	
Lampsilis siliquoidea	Ambleminae	7.7–13.0	
Lampsilis rafinesqueana	Ambleminae	5.5–6.0	
Venustaconcha ellipsiformis	Ambleminae	2.5–2.9	
Villosa iris	Ambleminae	8.8–9.6	Wang et al., 2007b

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