

## The invasive aquatic macrophyte *Hydrilla verticillata* facilitates the establishment of the invasive mussel *Limnoperna fortunei* in Neotropical reservoirs

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### ABSTRACT

The effects of introduced species on native species have been widely studied, however, invader-invader interactions are still poorly explored. Two non-native invasive species, the bivalve *Limnoperna fortunei* and the submersed macrophyte *Hydrilla verticillata*, have infested many aquatic ecosystems worldwide, and they are rapidly becoming nuisances in several water bodies in Brazil. We tested the hypothesis that *H. verticillata* facilitates the establishment of *L. fortunei* more than do native macrophytes. We surveyed mussels attached to two native macrophytes and to this invasive macrophyte in three Brazilian reservoirs and compared the length, density and biomass of the attached mussels relative to the host macrophyte species. All of the values of these attributes in *L. fortunei* were higher in the mussels attached to *H. verticillata* than in those attached to the native macrophytes. These results supported our initial hypothesis because the settlement of *L. fortunei* appeared to be facilitated by the invasive macrophyte, allowing higher abundances of the mussels as well as the more effective establishment of the mussel population. Various mechanisms could interact to facilitate the superior performance of *L. fortunei* in the invasive plant. *H. verticillata* is more flexible and withstands more mussels without breaking. Furthermore, this plant has different attached algae, which may be more beneficial for mussels. Thus, as a response to the facilitation suggested by our work, the mussel has greater opportunities to succeed in habitats colonised by the non-native *H. verticillata*. Although we have not evaluated the effects of this facilitation on aquatic communities or the ecosystem, our results could represent the first step of an invasional meltdown.

Key words: Co-occurrence, exotic species, *Egeria najas*; *Egeria densa*, positive interactions, non-native species.

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### INTRODUCTION

Several studies have investigated the changes caused by non-native invasive species in native populations, communities and ecosystems (Clayton and Edwards, 2006; Thouvenot *et al.*, 2012). Although several of these studies have found negative effects, caused by competition (Klein and Verlaque, 2009; Michelan *et al.*, 2010; Vilà *et al.*, 2011), others have found positive effects (Rodríguez, 2006; Altieri *et al.*, 2010), such as facilitation of native species (Schlaepfer *et al.*, 2011). As for invader-invader interactions, Green *et al.* (2011) have observed that they may have broad but still poorly explored consequences for invasion success, potentially affecting the evolution of species traits, the attributes of the recipient communities and propagule pressure.

Two non-native invasive species, the bivalve *Limnoperna fortunei* (Dunker, 1857), known as the golden mussel, and the submersed macrophyte *Hydrilla verticillata* L.f. Royle, commonly designated *hydrilla*, have infested many aquatic ecosystems worldwide and are becoming nuisances in several water bodies in Brazil (Sousa *et al.*, 2009; Sousa, 2011). *Limnoperna fortunei* is native to Southeast Asia. It is a sessile freshwater mytilid mussel that forms aggregations (druses), and it has been

introduced in other regions and has become troublesome (Ricciardi, 1998), especially in East Asia and North America (Ricciardi, 1998; Belz *et al.*, 2012). In Brazil, it was first recorded in 1999 (Mansur *et al.*, 1999, Takeda *et al.*, 2003). This species attaches to hard or soft and natural or artificial substrata (Darrigran and Ezcurra de Drago, 2000; Karatayev *et al.*, 2007), such as rock, plants, artificial plants, trunks, shells, piers, tubes and walls (Darrigran and Ezcurra de Drago, 2000; Karatayev *et al.*, 2007; Burlakova *et al.*, 2012). The second non-native species, *H. verticillata*, is a rooted-submersed aquatic macrophyte (family Hydrocharitaceae) native to Asia and many Pacific islands. It is now widespread throughout Australia as well as in scattered sites in Europe, South and North America and Africa (Cook and Lüönd, 1982; Madeira *et al.*, 2007; Sousa, 2011). This species has recently been introduced in several aquatic ecosystems in the Neotropics (Sousa *et al.*, 2009). *Hydrilla verticillata* can influence the morphology of freshwater ecosystems, increasing the physical complexity of the habitat. Because this species produces high biomass (Sousa *et al.*, 2010), it competes intensively with native species. *Hydrilla verticillata* is morphologically similar to two native species (*Egeria densa* Planch. and *Egeria najas* Planch.; Becker *et al.*,

2007). However, both species co-occur side by side in several Brazilian reservoirs in the Paraná river basin (T.S. Michelin, personal observation).

Aquatic macrophytes play an important role in structuring habitats in several aquatic ecosystems. These plants vary in their level of complexity and include a variety of life forms that occur along depth gradients, providing habitat for aquatic organisms and consequently influencing the attributes of their associated assemblages. Several studies have focused on the relationship between the associated fauna and the structural complexity of macrophytes (Jeffries, 1993; Pelicice and Agostinho, 2006). However, no studies to date have focused on the preferences of *L. fortunei* for establishment on non-native or native species of macrophytes or on understanding the mechanisms that influence invasive macro-invertebrates' community diversity, structure, and abundance when attached to these macrophytes (Ritchie and Olf, 1999; Almany, 2004; Taniguchi and Tokeshi, 2004).

In view of the effects of invasive species on native communities, we conducted this study to investigate whether certain submersed aquatic macrophyte species (invasive or native) facilitate the occurrence of an invasive mussel species. We hypothesised that *Hydrilla verticillata* facilitates the establishment of *Limnoperna fortunei* more than do native macrophyte species. This hypothesis assumes that both *H. verticillata* and *L. fortunei* are native to the same region and, thus, share a common evolutionary history.

## METHODS

### Sampling methods

We sampled the aquatic macrophytes in three reservoirs located in several rivers of the Upper Paraná river basin (the Paranapanema, Tietê and Paraná rivers). The native macrophytes examined were *E. densa*/*E. najas*, and the non-native invasive was *H. verticillata*. We selected three independent sampling stations per reservoir (located at distances greater than approximately 4 km from each other). We chose places where at least two species (the non-native and one or two natives) occurred simultaneously and nearby. The three species co-occurred at three sampling stations, *E. densa* and *H. verticillata* co-occurred at four stations, and *E. najas* and *H. verticillata* co-occurred at two stations. Thus, *H. verticillata* co-occurred with at least one native species at all sampling stations.

To evaluate whether the invasive macrophyte *H. verticillata* facilitated the mussel more than did the natives, we sub-sampled five individuals (each approximately one meter in length) of each macrophyte, including one main stem and roots, at all sampling stations. The individuals were placed in a plastic bottle with 70% alcohol for sub-

sequent screening of the *L. fortunei* and to measure the dry weight of the macrophytes.

In the laboratory, we separated individual *L. fortunei* from the plants under a stereoscopic microscope, and the following attributes of the mussel were measured: length (cm), number of individuals and bivalve biomass per macrophyte biomass (dry weights - DW). We measured mussel length in randomly selected 20 individuals per sample station. The DWs of both bivalve and macrophyte were obtained after drying the material in an oven at 80°C. Because we collected five fragments at each sampling station, the means of the variables above, obtained for all five plants, were considered replicates in the analyses.

### Data analysis

We applied a Student's *t*-test for independent samples to evaluate differences in the attributes of *L. fortunei* (length, density and biomass of individuals per macrophyte DW) between invasive and native macrophytes. Because the three species did not co-occur at all sites, we grouped the results for both species of *Egeria* spp. for comparison with the results found in *H. verticillata*. Accordingly, our results expressed differences between the invasive species and the native macrophytes. The homoscedasticity of the data was tested using a Levene test. If this assumption was not satisfied by the data, we used a nonparametric Mann-Whitney U-test. We used the program STATISTICA 7.0 (StatSoft, Inc. 2007) to perform all analyses.

## RESULTS

The plant biomass was, on average 2.04 g ( $\pm 0.39$  SD) for *H. verticillata* and 2.41 g ( $\pm 0.40$  SD) for *Egeria* spp and they are not different significantly from each other ( $t=-1.64$ ,  $df=19$ ,  $P=0.12$ ). All *L. fortunei* attributes (length, density and biomass per macrophyte DW) were higher for the bivalves colonising *H. verticillata* than for those attached to the native macrophyte species (Fig. 1).

The lengths of the mussels varied from 0.45 to 2.1 cm, and the Student *t*-test results showed that these lengths differed significantly between the invasive and native macrophyte species ( $t=6.88$ ,  $df=19$ ,  $P<0.001$ ; Fig. 1A). *Hydrilla verticillata* was the plant that showed the largest individuals of *L. fortunei*.

The mussel densities varied from 0 to 82.44 ind. g DW<sup>-1</sup>, and the mussel biomass varied from 0 to 0.75 g DW<sup>-1</sup>. The invasive mussel's density and biomass were both significantly affected by the macrophyte species (Mann-Whitney test -  $Z=3.77$ ,  $P<0.001$  and  $Z=3.84$ ,  $P<0.001$ ; Fig. 1 B,C). The mean values of these two response variables were higher for the bivalves attached to *H. verticillata* than for those attached to the native macrophytes (Fig. 1 B,C).

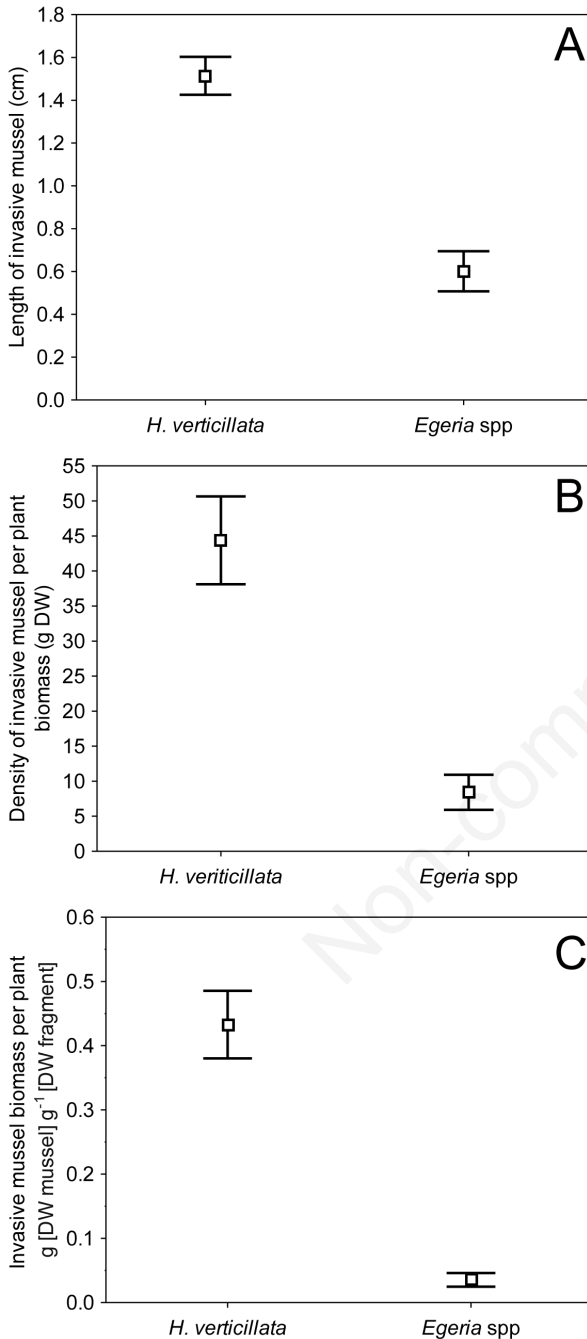
## DISCUSSION

Our study showed that the values of all attributes (length, density and biomass of *L. fortunei*) were higher in the invasive macrophyte (*H. verticillata*) than in the na-

tive species (*E. densa* or *E. najas*). These results support our hypothesis that the settlement of *L. fortunei* is facilitated by the invasive macrophyte. *Hydrilla verticillata* supports higher abundances of the invasive mussels as well as more successful establishment of their populations, with larger individuals than those associated with native plants. Note that, although *H. verticillata* is one of the most extensively studied aquatic plants worldwide, knowledge about ecological processes involving this species is still lacking (Sousa, 2011). To our knowledge, the current study is the first to address the specific association between *H. verticillata* and *L. fortunei*, both troublesome non-native invaders in freshwater ecosystems worldwide.

*Hydrilla verticillata* is similar in morphology with *Egeria* sp. (Sousa, 2011). This observation might appear to imply that the relationships of these plants to attached invertebrates would not differ. However, *H. verticillata* is more flexible and resistant to flow than the two native species considered in this study (Sousa, 2011). For this reason, it is, most likely, able to support more individuals and biomass of *L. fortunei* without breaking. Indeed, the upper shoots of this species withstand drag forces for a substantially longer time than other submersed species (Zhu *et al.*, 2012). In addition, *H. verticillata* has small teeth on its surface (Langeland, 1996), which make it rough to the touch, whereas *Egeria* spp. do not have these teeth; surface rugosity may be important in determining the density and size of attached invertebrates (Downes *et al.*, 1998). Thus, the differences that we found can be explained by physical features (in terms of resistance to breakage) together with the higher leaf rugosity of *H. verticillata*. Note that the apparently greater facilitation of *L. fortunei* provided by *H. verticillata*, compared with that provided by native macrophytes, has occurred despite the very recent date of the introduction of *H. verticillata* and the bivalve in Brazil (less than 13 years ago; Sousa, 2011; Belz *et al.*, 2013). Thus, in addition to the physical features, we suspect that the evolutionary history shared by *H. verticillata* and *L. fortunei* may be important to explain our results because both species are native to Asia.

Furthermore, we suppose that other mechanisms (in addition to physical differences) could explain the superior performance of *L. fortunei* in the invasive plant compared with that in the native plants. Mormul *et al.* (2010b), for example, showed that the assemblages of epiphytic algae in *H. verticillata* and *E. najas* differ significantly. According to Lodge (1986), changes in attached microorganisms and organic matter can affect invertebrates. Although individuals of *L. fortunei* are filter-feeding organisms (Sylvester *et al.*, 2005), the differences between the loosely attached algal assemblages colonising *H. verticillata* and *E. najas* (Mormul *et al.*, 2010a), which are potentially used as food



**Fig. 1.** Presence of *Limnoperna fortunei* in the macrophyte species *Hydrilla verticillata* (invasive - N=9) and *Egeria* spp. (native - N=12). *Limnoperna fortunei* length (A), density (B, number of *L. fortunei*) and biomass (C, biomass of *L. fortunei*) per unit macrophyte biomass.

by the bivalve, could affect the colonisation by bivalves. In addition, there are evidences that the settlement of some bivalves increases in filamentous substrates (Folino-Rorem *et al.*, 2006). Thus, the above factors related with differences in filamentous algae colonizing the macrophytes that we analyzed here could have influenced the higher abundance and especially the larger sizes of *L. fortunei* individuals in *H. verticillata*.

*Limnoperna fortunei* does not colonise substrates like mud, it needs a relatively consolidated substrate for its establishment (Morton *et al.*, 1973). *Hydrilla* and *Egeria* spp. banks may structure habitats, provide adequate colonisation sites and increase the attachment surface for this non-native invasive mussel. Although these species of macrophytes co-occur in several reservoirs in the Paraná River Basin, such as those that we investigated here, their distribution differs in natural habitats. In a floodplain of the Upper Paraná River (located downstream from the reservoirs where we collected our samples), for example, native macrophytes colonise floodplain lakes (Sousa *et al.*, 2010) and are scarce in the river and in its lateral channels (Sousa *et al.*, 2009; Sousa, 2011), where *H. verticillata* attains high densities and biomass (Sousa *et al.*, 2009). Thus, because *H. verticillata* offers a more suitable colonisation site for *L. fortunei*, as suggested by our results, we suppose that the main river channels and its lateral arms are more prone to bivalve proliferation than natural floodplain lakes, where *H. verticillata* does not grow.

Finally, our results suggest that the arrival of *H. verticillata* in the Paraná basin in 2005 (Sousa, 2011) helped to facilitate the success of *L. fortunei* in this basin. If so, we might have detected at least the first step of an *invasional meltdown* (Simberloff and Von Holle, 1999) in the ecosystems we studied. We base this inference on the facilitation of the bivalve by the invasive macrophyte and on the superior ability of *L. fortunei* to filter water (Sylvester *et al.*, 2005), leading to cascade effects on the entire ecosystem. However, we emphasise that a full *invasional meltdown* was not demonstrated in our work and that further studies are necessary to better assess this process.

## CONCLUSIONS

We confirmed our hypothesis that the invasive macrophyte *H. verticillata* enhanced the performance, as suggested by individual attributes, of the invasive bivalve *L. fortunei*. If the facilitation of bivalves by *H. verticillata* is greater than that provided by native macrophytes, as our results indicate, then the invasive macrophyte helps to increase the successful colonisation and spread of the mussel. Further studies of the interaction between these exotic species should incorporate the measurement of dynamic variables, including the growth rates and reproduction of the bivalve.

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