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JÉSSICA ERNANDES DA SILVA

Limnoperna fortunei **(Dunker, 1857) larvae:** from abiotic filters to potencial impacts on phytoplankton community

Maringá
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Tese apresentada ao Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais do Departamento de Biologia, Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutora em Ecologia e Limnologia.
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Larvas de *Limnoperna fortunei* **(Dunker, 1857**): dos filtros abióticos aos impactos potenciais sobre a comunidade de fitoplâncton

RESUMO

O sucesso das invasões biológicas depende da superação filtros ambientais. Após a transposição dos filtros e consequente estabelecimento, as espécies invasoras podem atingir altas densidades e ocasionar impactos, como perda de biodiversidade e prejuízos ao funcionamento dos ecossistemas. O mexilhão-dourado (*Limnoperna fortunei*) é um bivalve invasor na América do Sul e apresenta elevadas densidades nos ambientes invadidos. Apresenta fase larval planctônica, dividida em cinco estágios, e adulta incrustante. A fase larval é considerada o principal propágulo da espécie. É sabido que os fatores abióticos atuam diferentemente sobre os estágios larvais, dado que estes apresentam diferenças morfológicas e fisiológicas. Alguns trabalhos evidenciaram a capacidade de *L. fortunei* em selecionar grupos e traços funcionais das espécies de fitoplâncton, porém esses estudos são experimentais e com representantes adultos. Considerando que as larvas são o principal propágulo da espécie, logo, os filtros abióticos agem primariamente sobre elas e buscou-se (i) avaliar a estruturação populacional das larvas de *L. fortunei* e sua relação com os fatores ambientais em ambientes lóticos da planície de inundação do alto rio Paraná. Ainda, considerando que as larvas podem atingir elevados valores de densidade também buscou-se (ii) identificar os potenciais impactos das altas densidades larvais sobre a diversidade taxonômica e funcional de fitoplâncton, e seus reflexos sobre o funcionamento ecossitêmico. Os resultados evidenciaram diferenças na pirâmide etária entre os ambientes avaliados, e constatou-se que a maioria dos filtros ambientais age sobre os estágios larvais iniciais. Dentre esses filtros, destaca-se a turbidez. Também identificou-se o efeito negativo da densidade larval de *L. fortunei* sobre a diversidade funcional e taxonômica da comunidade de fitoplâncton. Os efeitos da densidade larval sobre essas facetas da diversidade do fitoplâncton resultaram em efeitos negativos indiretos sobre o estoque de biomassa da comunidade desses produtores. Espera-se que o conhecimento sobre os filtros que controlam as densidades larvais de *L. fortunei* possa contribuir para o controle e manejo da espécie, a fim de evitar maiores impactos sobre o fitoplancton, e consequentemente, sobre o ambiente.

Palavras-chave: Estrutura etária. Filtros ambientais. Diversidade funcional. Mexilhão-dourado. Espécie invasora.

Limnoperna fortunei **(Dunker, 1857) larvae**: from abiotic filters to potencial impacts on phytoplankton community

ABSTRACT

The success of biological invasions depends on overcoming environmental filters. After the filters are transposed and subsequently established, invasive species can reach high densities and cause impacts, such as loss of biodiversity and damage to the functioning of ecosystems. The golden mussel (*Limnoperna fortunei*) is an invasive bivalve in South America and presents high densities in invaded environments. It has a planktonic larval stage, divided into five stages, and an encrusting adult. The larval stage is considered the main propagule of the species. It is known that abiotic factors act differently on larval stages, as these present morphological and physiological differences. Some works shown the ability of *L. fortunei* to select groups and functional traits of phytoplankton species, but these studies are experimental and with adult representatives. Considering that larvae are the main propagule of the species, therefore, abiotic filters act primarily on them, we sought to (i) evaluate the population structure of *L. fortunei* larvae and its relationship with environmental factors in lotic environments in the floodplain of the Upper Paraná River. In addition, considering that larvae can reach high density values, we also sought to (ii) identify the potential impacts of high larval densities on the taxonomic and functional diversity of phytoplankton, and their effects on ecosystem functioning. The results showed differences in the age pyramid between the evaluated environments, and found that most environmental filters act on the early larval stages. Among these filters, turbidity stands out. We also identified a negative effect of *L. fortunei* larval density on the functional and taxonomic diversity of the phytoplankton community. The effects of larval density on these facets of phytoplankton diversity resulted in indirect negative effects on the biomass stock of the community of these producers. It's expected that knowledge about the filters that control larval densities of *L. fortunei* can contribute to the control and management of the species, in order to avoid major impacts on the phytoplankton, and consequently, on the environment.

***Keywords*:** Age structure. Environmental filters. Functional diversity. Golden mussel. Invasive species.

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SUMMARY

1 INTRODUCTION

Biological invasions are considered one of the main threats to biodiversity (Kipp et al., 2010, Linders et al. 2019), given it has been related to several extinction events of native species worldwide (Bellard et al., 2016). Species invasions begin with the transport of organisms, whether intentionally or accidentally (Colautti & McIsaac, 2004; Henderson et al., 2006), from their native habitats to new environments. These processes of dispersion of organisms have been facilitated by globalization (Meyerson and Mooney, 2007). For the invasion process to be successful, in addition to dispersal, the invasive species must overcome environmental and biotic filters imposed by the invaded environment (Havel et al., 2005; Lockwood et al., 2009; Gama et al. 2017).

Aquatic ecosystems are sensitive to species invasion, as they face constant anthropogenic impacts, such as the construction of reservoirs (Couto & Olden, 2018) and eutrophication processes (Picart et al., 2015). In addition, these environments are high connected, which facilitates the dispersal step, and consequently, spread of invasive species between adjacent environments (Meghan et al., 2018; Amo et al., 2021).

Among the filters environmental, the abiotic stands out primarily for the successful establishment of the invasive species, given that their survival depends on local abiotic conditions (Von Holle & Simberloff, 2005; Lewis et al., 2017). In aquatic ecosystem, the abiotic factors, such as temperature, pH, and oxygen play a key role affecting (negatively or positively) the invasive species (Oliveira et al., 2010; Fey & Herren, 2014; Ernandes-Silva et al., 2016). Additionally, the biotic interactions in a new environment may also be important and limit invasion success (Rahel 2002). For example, in aquatic systems, phytoplankton is one of the main food sources for filter feeding organisms, such as mussels (Jeppesen et al. 1996). Therefore, the composition of this community can affect the establishment of these organisms. On the other hand, the high densities of the invader also have impacts on the local community (Lockwood et al., 2013; Simberloff et al., 2013; Bellard et al., 2016).

Limnoperna fortunei, popularly known as golden mussel, is an invader of Asian origin that has caused several impacts across Southeast Asia and South America, such as clogs water supply infrastructures, and impairing the functioning and dynamics of the trophic chain of freshwater ecosystems (Boltovskoy & Correa, 2015; González-Bergonzoni et al., 2020). Multiple biological features contribute to the success of *L. fortunei* invasions, such as rapid growth and maturation, and colonization ability (Darrigran et al., 1999; Giglio et al., 2016). Some intrinsic characteristics of its life cycle, such as the planktonic larval stage and fouling adult, can facilitate its dispersion between environments, given that the larval stage of the *L. fortunei* is easily dispersed by running water, while the adult stage is dispersed by the traffic of colonized vessels (Boltovskoy et al., 2006). Thus,

the larval stages are considered the main propagule source of the *L. fortunei* and its release can coincide with the flood period (Ernandes-Silva et al., 2016), which facilitates your entry into new environments. The larvae stages are classified in five categories (Santos et al., 2005): D-shaped larval, straight-hinged veliger, umbonated-veliger, pediveliger and plantigrades (Fig 1). Each larval stage may be affected by different environmental filters (Ernandes-Silva et al., 2016), as they present morphological and physiological differences among themselves (Santos et al., 2005).

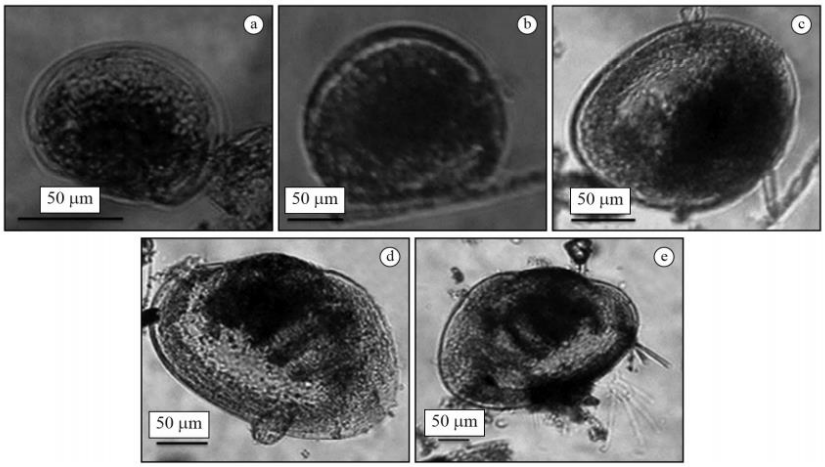

Figure 1. Larval stages of *Limnoperna fortunei*. A: D-shaped larval, B: straight-hinged veliger, C: umbonated-veliger, D: pediveliger and E: plantigrades. Image taken from Ernandes-Silva et al, 2016.

Among the impacts resulting from the density of *L. fortunei*, we can highlight its effects on the of phytoplankton communities (Boltovskoy & Correa, 2015), which may reflect on ecosystem changes since phytoplankton is one the main primary producer of these ecosystems (Field et al., 1998). However, studies with this focus have been experimental, requiring investigations in natural environments. The larval stages of *L. fortunei*, despite being small (90 µm - 490 µm) can reach high densities (around 2,000 ind. m^{-3}, Oliveira et al., 2011), and thus, cause impacts on the invaded environment, especially on the community of resources such as phytoplankton.

In this context, we carried out two studies in which were evaluated (i) the population structure of the larvae of *L. fortunei* in lotic environments of the upper Paraná River floodplain and its relationship with the local environmental variables; and (ii) the impacts of larval density on the functional and taxonomic diversity of the phytoplankton community, and its potential effect on biomass stocks, in lotic environments of the upper Paraná River floodplain.

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2 FACTORS ASSOCIATED WITH THE POPULATION STRUCTURE OF AN INVASIVE MOLLUSC IN A FLOODPLAIN NEOTROPICAL

ABSTRACT

Limnoperna fortunei is an invasive mussel species that is continuously expanding through South American. It has five larval stages, which usually differ in tolerance to environmental factors. How different abiotic filters affect *L. fortunei* larval stages, has not been studied in depth. We employed a detailed database describing five floodplain environments to investigate the distribution of *L. fortunei* larval stages within and among these environments and determine which local abiotic filters affect the density of these larval stages. We found that the two youngest larval stages accounted for up to 83% of the larval density of *L. fortunei* in four of the five environments studied, evidencing an expanding population pyramid of *L. fortunei* in these environments. We also found positive and negative relationships among abiotic filters, and these relationships strongly affect only the density of the youngest larval stages. Turbidity, water level, and suspended inorganic matter directly and negatively affected the density of the two youngest larval stages. Conversely, temperature and pH directly and positively affected the density of D-shaped and umbonated, respectively. Additionally, water level indirectly increased the density of the youngest larval stages mediated by a decrease in turbidity and suspended inorganic matter. Our findings suggest a likely expansion of *L. fortunei* along the Upper Paraná River Floodplain. However, the abiotic filters, such as turbidity, water level, and suspended inorganic matter decrease the density of the youngest larval stages, indicating that establishment control of *L. fortunei* may occur in these larval stages.

Keywords: abiotic filters; Bivalvia, *Limnoperna fortunei;* larval stages; invasive mussel.

2.1 Introduction

Understanding the pathways by which introduced species establish and become invasive is crucial to anticipate new invasions and control invasive species (Simberloff 2011). However, for successfully establish yourself in a new environment, non-native species are directly dependent on the local abiotic filters (Von Holle and Simberloff 2005; Lewis et al. 2017). Despite the importance, abiotic filters have been less studied under an invasion perspective than the filters related to dispersal and biotic relationships with native species (Seebens et al. 2015; Zwerschke et al. 2018). This represents a critical gap in the knowledge regarding invasion in aquatic environments, since abiotic filters, such as temperature, pH, oxygen, turbidity, and water level play a key role explain the establishment of non-native species (Oliveira et al. 2011; Ernandes-Silva et al. 2016; Amo et al. 2021). In addition, the abiotic filters may allow that some sites having a greater invasibility than others (Amo et al. 2021).

Floodplains are complex aquatic systems composed of different types of environments (Agostinho et al. 2004a). In these systems, the flood pulse increases the connectivity among environments, which favors the dispersion of non-native propagules (Amo et al. 2021). Thus, floodplain systems may be highly susceptive to invasion by non-native species, mainly those with planktonic life forms, such as the Asian golden mussel *Limnoperna fortunei* (Amo et al. 2021). *Limnoperna fortunei* (Dunker, 1857) (Bivalvia: Mytilidae) is a native species from mainland China that was probably introduced in South America by vessel ballast water from commercial ships (Boltovskoy et al. 2006). Since then, *L. fortunei* has colonized and invaded many environments in South America including the Upper Paraná River floodplain (Ernandes-Silva et al. 2016; Amo et al. 2021). Previous studies have shown that *L. fortunei* should be able to colonize other water systems across the globe in the coming decades (Souza Campos et al. 2014; Petsch et al. 2020). This is extremely worrisome since *L. fortunei* has caused pervasive impacts across Southeast Asia and South America, such as clogged water supply infrastructures and impaired the structure and functioning of aquatic ecosystems (Boltovskoy and Correa 2015; Boltovskoy 2015).

The successful invasion of *L. fortunei* in freshwater systems may be explained by biological characteristics of the species, such as short life cycle, high fecundity, rapid growth, and high ability to disperse and colonize new sites (Darrigran et al. 1999; Giglio et al. 2016). Regarding the dispersion, the larval stages of *L. fortunei* are easily dispersed by running water, whereas the adult stage is dispersed by the traffic of colonized vessels, sports fishing boats, and live fish (Boltovskoy et al. 2006; Boltovskoy 2015). In floodplain systems, the dispersion of larval stages of *L. fortunei* is directly dependent on the water level fluctuations and occurs during flood periods, in which larval are release and dispersed to several environments (Amo et al. 2021).

Previous studies have identified multiple local abiotic filters that may affect the establishment of adult stages *L. fortunei* (e.g., Oliveira et al. 2011; Linares et al. 2020). However, like these local abiotic filters affect the different larval stages of *L. fortunei*, has not yet been studied with more details (see, Ernandes-Silva et al. 2016; Amo et al. 2021). The larval stages of *L. fortunei* are classified into five categories and abiotic filters may act differently upon the occurrence of each category (Ernandes-Silva et al. 2016) due to distinct morphological and physiological tolerances among larval stages (Santos et al. 2005). For instance, the occurrence of the youngest larval stages (i.e., D-shaped larval, straight-hinged veliger, and umbonated-veliger) seems to be most affected by abiotic filters, such as turbidity, suspended inorganic matter, conductivity, and pH (Ernandes-Silva et al. 2016). High turbidity and suspended inorganic matter decrease the occurrence of the youngest larval stages because impairs the filtration of these bivalves (Tokumon et al. 2015; Ernandes-Silva et al. 2016). Likewise, high acidity (low pH) limits the valve structure formation of *L. fortunei*, which occurs during the youngest larval stages (Ezcurra de Drago et al. 2009). By contrast, the high temperature seems to increase the occurrence of youngest larval stages of *L. fortunei*, since increases the reproduction rate of adult, consequently youngest larval are released into the environment during warming periods (Boltovskoy 2015). The oldest larval stages (pediveliger and plantigrades) are weakly affected by abiotic filters, but their occurrence may be affected by food availability (e.g., phytoplankton biomass; Ernandes-Silva et al. 2016). Although it has been proposed that local abiotic filters have greater effects on the youngest larval stage and that larval stages are affected by distinct abiotic filters (Ernandes-Silva et al. 2016), we know little about how different abiotic filters interact to influence, direct and indirect, the density of each larval stages of *L. fortunei*.

In this study, we used a database holding data for 2-years from five tropical floodplain environments (three rivers and two channels). We aimed to investigate the density of the larval stages of *L. fortunei* among the environments. We also compared the percentage of the larval stages of *L. fortunei* within each environment where the *L. fortunei* was sampled. Furthermore, we also investigate the relationship between multiple local abiotic filters (see, methods) and how these relationships could affect the density of the different larval stages of *L. fortunei*. We predicted that (i) the density of larval stages of *L. fortunei* will differ between environments depending on the local abiotic filters within these environments; (ii) abiotic filters effects would be stronger on the density of the youngest than oldest larval stages; (iii) there are relationships among abiotic filters, which indirectly would decrease the density of the larval stages, mainly youngest.

2.2 Materials and Methods

2.2.1 Study area

The study was conducted in the Upper Paraná River floodplain (22 º46´27.53"S and 53º 19´ 57.95"W), Brazil South, America (Fig. 1). This region has a tropical climate with a mean annual temperature of 22 °C (mean minimum and maximum temperatures of 10.3 and 33.6 °C, respectively) and mean annual precipitation of 1500 mm. The data used in the study are part of a "long-term ecological research project" (PELD-Sitio PIAP). Data were collected for 2-years (2011-2012), four annual samples (conducted in the four seasons of the year; March, June, September, and December) were taken at the same time in five different environments (Baía River, Ivinhema River, Paraná River, Curutuba Channel, and Ipoitã Channel; Fig. 1). These five environments differ in water flow, depth, and other physical and chemical variables, which characterize it as having great environmental heterogeneity (Roberto et al. 2009). For instance, the five environments vary in the degree of human impacts, being the Paraná River and Ipoitã Channel most impacted due to dams (Agostinho et al. 2004a). The Baía River and Curutuba Channel are affected by livestock activity on its margins (Agostinho et al. 2004a), and the Ivinhema River being the most preserved environment as it is located in an area of permanent preservation (Braghin et al. 2018).

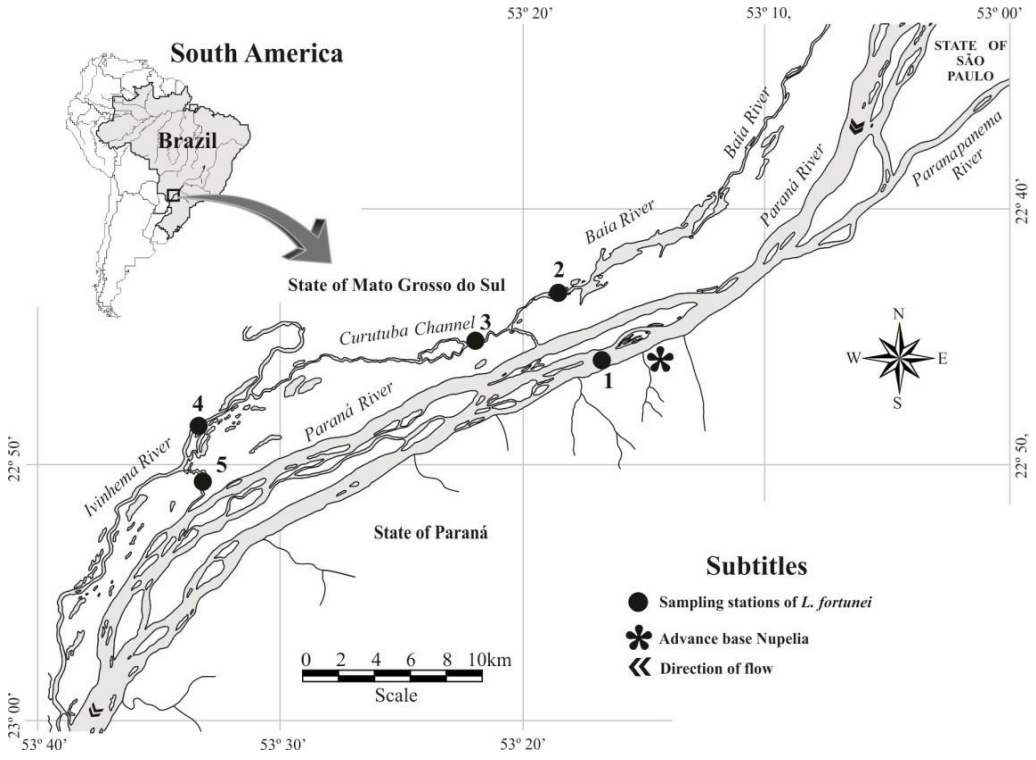

Figure 1. Study area with sampling stations. 1: Paraná River, 2: Baía River, 3: Curutuba Channel, 4: Ivinhema River, 5: Ipoitã Channel.

2.2.2 Samplings of *L. fortunei*

During the sampled period (see, above), to measure the larval density in each stage of *L. fortunei*, we obtained three samples in each environment by filtering water 100 L (totalizing 300 L for each environment per period) using buckets and a plankton net (30 μm mesh), totaling 120 samples. The samples were preserved in an 80% alcohol solution and the larvae were counted in the laboratory using an optical microscope. The larval stages were classified into five categories based on length (see Santos et al. 2005): D-shaped larval (90-130 μm; initial stage), Straight-hinged veliger (140-180 μm; initial stage), Umbonated-veliger (190-220 μm; intermediary stage), Pediveliger (230-270 μm; intermediary stage) and Plantigrades (280-490 μm; final stage). The individual densities of the larval stages were measured per cubic meter (i.e., ind. m^{-3}).

2.2.3 Samplings of environmental filters

Simultaneously to *L. fortunei*, in each sampled site we also sampled the phytoplankton community at the sub-surface in the pelagic zone using bottles and preserved in 10% acetic acid (Bicudo and Menezes 2006). Phytoplankton was counted using an inverted microscope according to the American Public Health Association-APHA (1985) and identified at the lowest possible taxonomic level (species) according to the specialized literature (Raviers 2006; Komárek and Anagnostidis 2005). We identified the nanoplankton algae (< 60 μm) and calculated the biomass of these algae based on their geometric form. We choose to use only nanoplankton species because these algae are the most filtered by *L. fortunei* larvae (Santos et al. 2005). Moreover, we also measured *in situ* the water temperature (ºC), percentage of dissolved oxygen (mg l^{-1}), pH, turbidity (NTU), water level (m), and samples water were collected and after in laboratory were measured: suspend inorganic matter (mg l^{-1}), total nitrogen (TN) and total phosphorus (TP). These variables were selected because often act as abiotic filters affecting the establishment of the adult stages of *L. fortunei* (Oliveira et al. 2011; Darrigran et al. 2012). Moreover, previous studies have shown that these abiotic filters also may influence the probability of occurrence of the larval stages of *L. fortunei* (Ernandes-Silva et al. 2016; Amo et al. 2021). Dissolved oxygen, pH, and turbidity were estimated using an oximeter (Digimed), portable potentiometer, and turbidimeter, respectively. To measure TP and TN, we collected water samples in each environment. TN was analyzed via the persulphate method (Bergamin et al. 1978) and determined in a spectrophotometer in the presence of cadmium, using a flow-injection system (Giné et al. 1980). TP was measured according to Golterman et al. (1978). The suspended inorganic matter was estimated by water filtration in GF 52 / C, 47 mm filters and subsequently incinerated at 470 ºC and weighed, according to Teixeira et al. (1965).

2.2.4 Data analysis

We evaluated the difference in density of the larval stages of *L. fortunei* among the five studied environments, and the difference in the percentage of the larval stages within each environment by using one-way ANOVA. When the ANOVAs were significant, we applied Tukey's HSD post hoc tests. ANOVA residuals were inspected for normality and homogeneity using Shapiro–Wilk and Levene's tests, respectively. We log-transformed the larval stage density to meet the ANOVA assumptions. Tukey's HSD test was performed using the *glht* function in the 'multcomp' package (Hothorn et al. 2013).

We employed piecewise structural equation modeling (Lefcheck 2016) to investigate the relationship among local abiotic filters (see methods above) and how they affect the density of the larval stages of *L. fortunei*. To carry out the piecewiseSEM, we specified an a priori model of relationships among all abiotic filters based on our ecological knowledge and previous studies' results (Fig. S1). Thus, the relationships among abiotic filters were based on what commonly is found in observational and experimental studies (Fig. S1). We highlighted that, there was little information on the effects of these abiotic filters on larval stages of *L. fortunei* (see, Ernandes-Silva et al. 2016; Amo et al. 2021). Moreover, to the best of our knowledge, no study investigates how the relationships among abiotic filters influence the density of the larval stages.

We tested multicollinearity for each trophic group by calculating the variance inflation factor (VIF). VIF > 3 indicates possible collinearity, which was not observed in our model. As we had many abiotic variables, we reduced the number of these variables in the piecewiseSEM using Akaike information criteria corrected for a small sample size (AICc), which is implemented in the piecewiseSEM package (Lefcheck 2016). This model selection resulted in more straightforward and more robust models to test how the relationships among abiotic filters affect the density of the different larval stages of *L. fortunei*. Then, the full model (including all abiotic filters) was compared with the reduced model (without some abiotic filters) using AICc (AICfullmodel - AICreducedmodel; Table 2). We used the lack of effect (direct or indirect) on the density of the larval stages as a criterion to remove any abiotic filter from the model. We considered ΔAICc > 2 units to distinguish the full model from the reduced models. Importantly, the full and reduced final model differed in at least ΔAICc = 206.34 units (Table S3). The pSEM was fitted using a linear mixed-effect model in the 'NLME' package (Pinheiro et al. 2016), which is implemented in the 'piecewiseSEM' package (Lefcheck 2016), with the seasons nested with each of five environments (three rivers and two channels) as a random factor. We present the standardized coefficient for each path and estimated the indirect effects by coefficient multiplication. Path significance was obtained by maximum likelihood and model fit was evaluated using Shipley's test of d-separation through

Fisher's C statistic ($P > 0.05$ indicates fitted model, i.e., there are no missing paths). We addressed any potential temporal autocorrelation among sampling periods using a continuous autoregressive 1 autocorrelation structure from the CAR 1 function in the 'NLME' package. Our analyses were conducted using R language.

2.3 Results

2.3.1 Larval distribution among and within of the environments

Over two years, the larval density in the five sampled environments was more than 80,286 ind. m^{-3} of *L. fortunei*. The two youngest larval stages accounted for 83% of the total sampled larval, while the two oldest larval stages accounted only for 3% of sampled larval (Fig. 2). Moreover, the percentage of the youngest larval stages was higher in December, coinciding with the reproduction period of *L. fortunei* (Fig. S2). The density of all larval stages was significantly different among five environments ($P < 0.05$; Table 1). Particularly, the two youngest larval stages (D-shaped and Straight-hinged veliger) had lower densities in the Ivinhema River than in the other four environments (Fig. 3a, b; Table S1). Conversely, the two oldest larval stages (Pediveliger and Plantigrades) had higher densities in the Ivinhema and Paraná Rivers and the Ipoitã Channel than in the other environments (Fig. 3d, e; Table S1). The proportion of the five larval stages within each environment also was significantly different ($P < 0.05$; Table 2). In the Baía River, Paraná River, Curutuba Channel, and Ipoitã Channel there was a higher proportion of youngest larval stages (Fig. 4; Table S2), which accounted for 76.3%, 66.9%, 82.6%, and 72.3% of the total of larval collected in these environments, respectively (inserted pyramids, Fig. 4). On the other hand, in the Ivinhema River, there was a higher proportion of the two oldest larval stages (Fig. 4; Table S2), which accounted for 73% of the total of larval collected in this river (inserted pyramids, Fig. 4a).

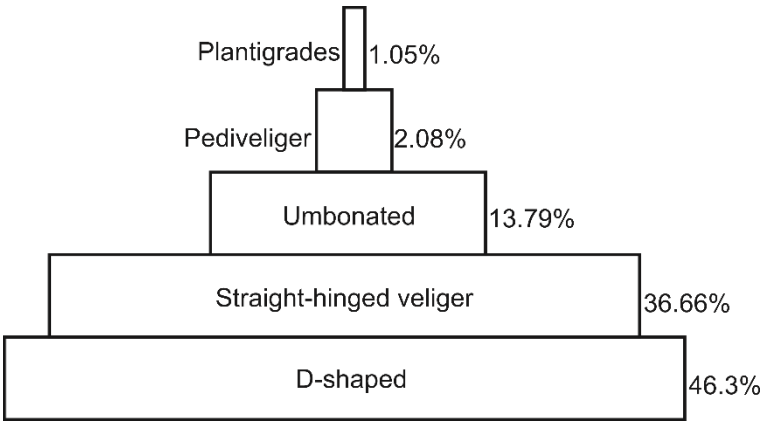

Figure 2. Population pyramid of *L. fortunei* across five studied environments. The values on the right represent the mean percentage of each larval stage during two years across. Note that, the *L. fortunei* have a typical expansive population pyramid as the youngest larval stages are most abundant than the oldest larval stages.

Table 1. Results of the analysis of variance (one-way ANOVA) testing effects of the environments on the density of the five larval stages of *L. fortunei*. Results of the Tukey posthoc test are shown in Fig. 3 and pairwise comparison among environments in Table S1.

Larval stages	Environments			
	df	Means of Squares	*F*	*P*
D-shaped	4	4.705	8.316	< 0.001***
Straight-hinged veliger	4	0.629	11.75	< 0.001***
Umbonated	4	4.557	12.79	< 0.0001***
Pediveliger	4	0.425	5.911	0.0021**
Plantigrades	4	1.899	28.91	< 0.0001***

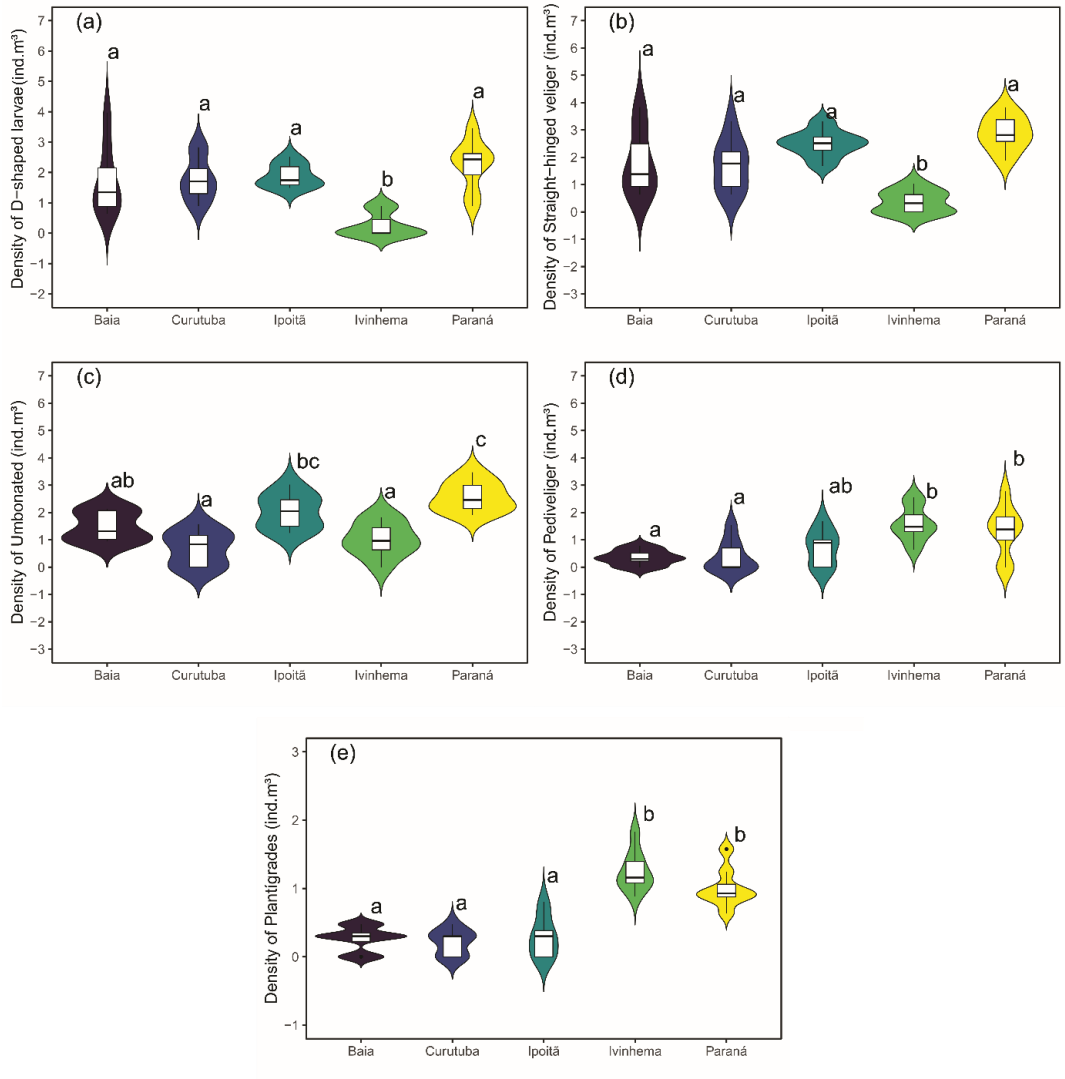

Figure 3. Violin plots comparing density of the larval stages of *L. fortunei* among five studied environments, being (a) D-shaped stage, (b) Straight-hinged veliger stage, (c) Umbonated stage, (d) Pediveliger stage, and (e) Plantigrades stage. Different lowercase letters within panels indicate significant ($P < 0.05$) differences between environments mean, after using Tukey posthoc test. Error bars represent ±1SE.

Table 2. Results of the analysis of variance (one-way ANOVA) testing the difference on proportion among larval stages of *L. fortunei* within each of the five environments. Results of the Tukey posthoc test are shown in Fig. 4 and pairwise comparison among larval stages to each environment in Table S2.

Larval stages	Larval stages of *L. fortunei*			
	df	Means of Squares	*F*	*P*
Baía River	4	2.894	8.062	< 0.001***
Curutuba Channel	4	4.634	10.72	< 0.001***
Ipoitã Channel	4	5.009	49.59	< 0.0001***
Ivinhema River	4	1.884	6.814	< 0.0001***
Paraná River	4	3.163	14.78	< 0.0001***

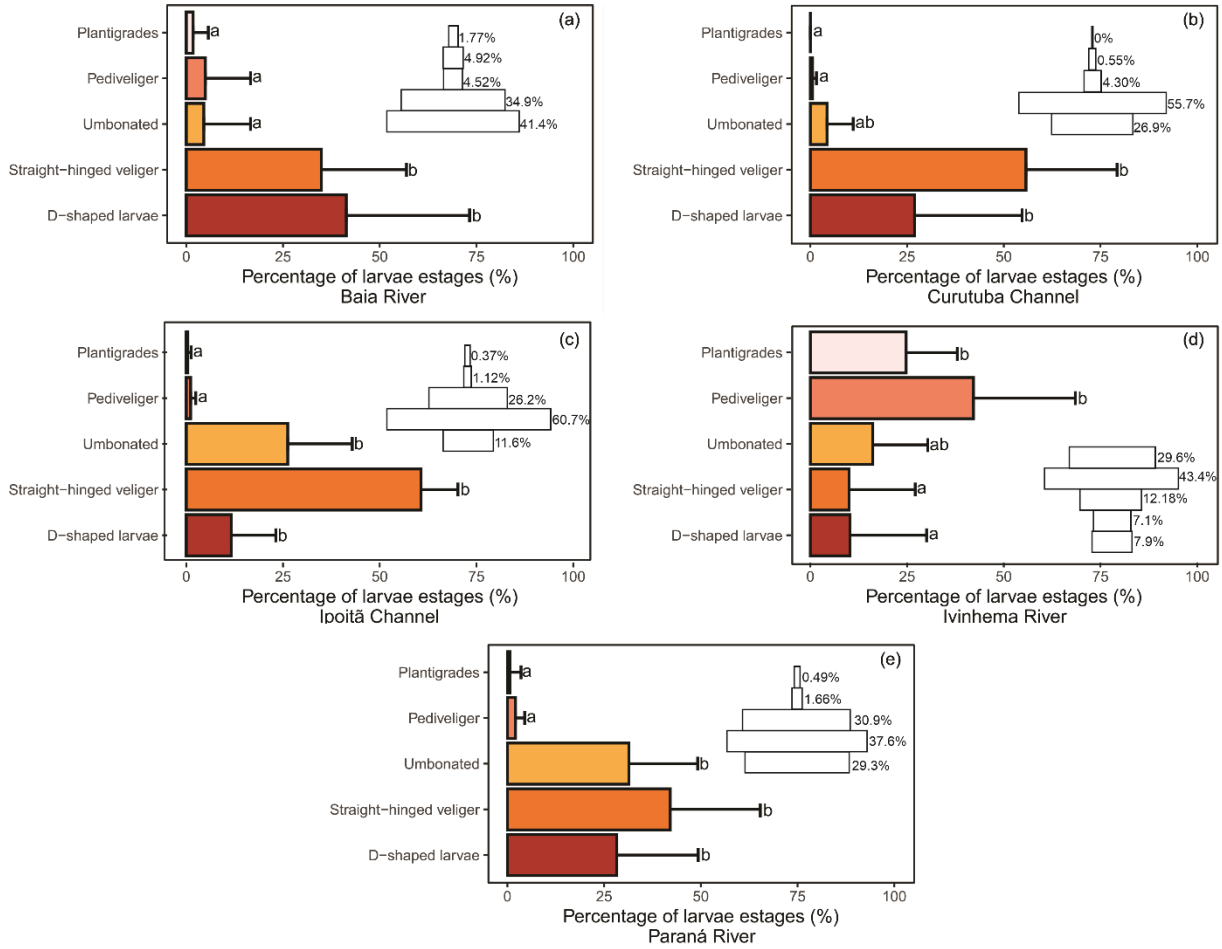

Figure 4. Percentage of the larval stages of *L. fortunei* within each of the five environments, being (a) Baía River, (b) Curutuba Channel, (c) Ipoitã Channel, (d) Ivinhema River, and (e) Paraná River. Different lowercase letters within panels indicate significant ($P < 0.05$) differences between environments mean, after using Tukey posthoc test. Error bars represent ±1SE. Inserted pyramids within each plot indicate the percentage among larval stages of *L. fortunei* within each environment.

2.3.2 Local abiotic filters affecting larval density

Structural equation modeling fit the data well (Fischer's C = 24.811, AICc = 120.811, P = 0.306) and revealed relationships among abiotic filters, which, direct and indirect, resulted in an

explanation of more than 60% of the variation in density of the five larval stages of *L. fortunei* (Fig. 5; Table S4). Directly, the abiotic filters, such as water level, turbidity, and the inorganic suspended matter had direct negative effects on the two youngest larval stages (Fig. 5). Conversely, the temperature had a direct positive effect on the D-shaped larval density, and the pH had a direct positive effect on the umbonated larval density (Fig. 5). Indirectly, the water level also had positive effects on densities of the two youngest larval stages (D-shaped and straight-hinged veliger) via negative effects on turbidity (r = 0.362; r = 0.211), and inorganic suspended matter, respectively (r = 0.142; r = 0.08; Fig. 5). By contrast, the suspended inorganic matter had strong negative indirect effects on D-shaped (r = -0.513) and straight-hinged veliger (r = -0.299) larval stages through positive effects on turbidity (Fig. 5).

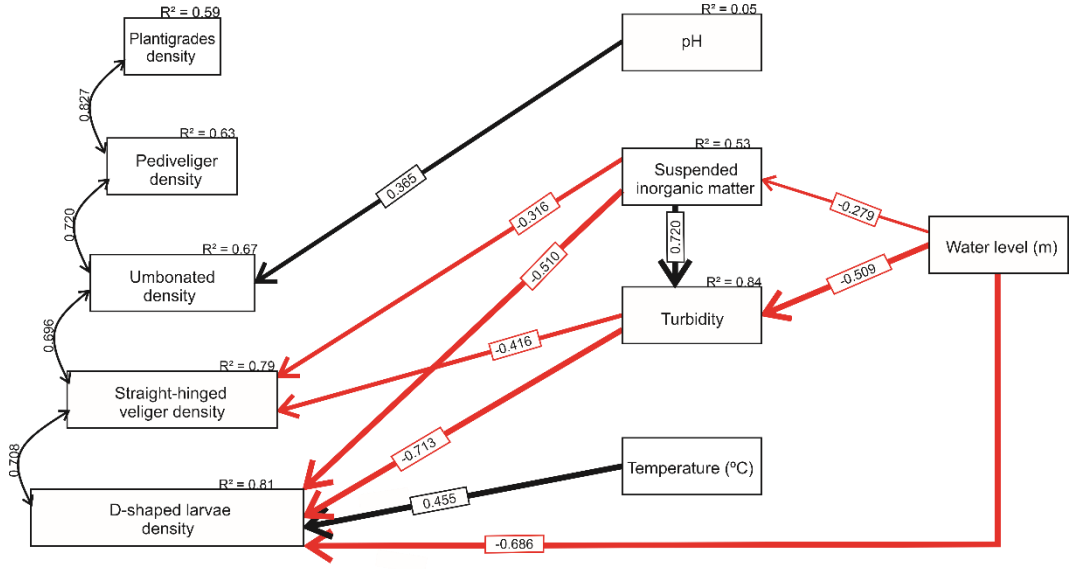

Figure 5. Structural equation models of the relationship between environmental predictors and each of the five larval *L. fortunei* stages. Solid black and red arrows represent significant positive and negative paths, respectively. Lines with double arrows indicate correlations between larval stages. R^2 for component models are given above the boxes of endogenous variables. To simplify the model's visualization the non-significant patches results were removed from the graphic, but are provided on the complete model's results (Appendix, Table S4).

2.4 Discussion

Identifying factors that affect the establishment of invasive species remains a challenging task in the field of applied ecology and ecosystem management. Here, we used a large-scale data set over a 2-years period to investigate the population structure of larval stages of the invasive golden mussel *L. fortunei* within and among different environments in a large floodplain system and evaluated which local abiotic filters affect the density of those different larval stages. Our results showed a higher density of the youngest larval stages in four of the five environments studied. In

these four environments, the youngest larval stages accounted for more than 80% of the larvae collected, suggesting that *L. fortunei* is expanding across the Upper Paraná River floodplain. Consequently, we found an expanding population pyramid of *L. fortunei* (i.e., broad base and narrow top; see Fig. 2), which occurs when there are many cohorts, and results in a higher density of younger individuals (e.g., Mouthon 2003). Indeed, *L. fortunei* is characterized by high and continuous reproduction mode (Boltovskoy 2015), and it may release thousands of larvae at a time (around 20,000 ind. m^{-3}), and in environmental conditions are favorable these larvae have a rapid growth, reaching maturity in about two weeks (Oliveira et al. 2011).

2.4.1 Larval density of *L. fortunei* within and between aquatic environments

An important detail is that our results demonstrated that the population structure of the larval stages was different within the five environments. For instance, there was a higher number of oldest larval stages in the Ivinhema River. On the other hand, this environment had a lower *L. fortunei* larval density compared to the other environments. In addition, turbidity and suspended inorganic matter were the main abiotic filters negatively affecting the density of the two youngest larval stages of *L. fortunei* in this environment. Therefore, turbidity and suspended inorganic matter likely play a key role in decreasing the density of the youngest larval stages in the Ivinhema River. Possibly these results are explained because, the Ivinhema River is more preserved than the other environments here studied because it is situated into a permanent preservation park (Braghin et al. 2018). Consequently, the Ivinhema River maintains the most pristine abiotic filters, such as high turbidity and suspended inorganic matter (Table S5), which are characteristic of the Upper Paraná River Floodplain (Agostinho et al. 2004a) and many other floodplains (da Cruz et al. 2021; Melack et al. 2021; Molinari et al. 2021; Nogueira et al. 2021).

In contrast, there was an expansive populational pyramid of *L. fortunei* in Baía and Paraná River, which are habitats more degraded by anthropogenic actions, for example, damming, overfishing, agriculture, and urbanization (Agostinho et al. 2004b; Braghin et al. 2018). As a result, the abiotic filters are weakened in these two rivers, e.g., there was low turbidity and suspended inorganic matter in Paraná and Baía River (see, Table S5). For instance, the Paraná River has several hydroelectric power plants built upstream, which have favored the establishment of many non-native species of fish (Moi et al. 2021) and macrophytes (Sousa et al. 2010). Furthermore, hydroelectric power can decrease the turbidity, suspended inorganic matter (Roberto et al. 2009; Moi et al. 2020) and our results suggest that this should also favor the establishment of *L. fortunei.*

The high density of the oldest larval stages (despite the low density of the youngest larval stages) in the Ivinhema River indicates that this river receives larval propagules, which likely come

from the Baía and Paraná River. Both Baía and Paraná River have a high density of youngest larval stages, but a low density of the oldest larval stages, which may be explained by three pathways: first, the oldest larval stages die inside the Paraná and Baía River; second, the larvae have settled on the substrate and are no found in the water column, and third, they are taken to other environments (e.g., Ivinhema River) via water flow. The high density of the intermediate larval stage (Umbonated) in the Curutuba and Ipoitã Channels, which connect the Baía and Paraná River to the Ivinhema River, respectively, reinforce the idea that larvae of *L. fortunei* in the Parana and Baía River are dispersed into the Ivinhema River. In addition, the main abiotic filters recorded at the Invinhema River, such as turbidity and suspended inorganic matter seem to have no effect on the oldest larval stages, which can thus survive in this environment.

2.4.2 Effects of the abiotic filters on larval stages of *L. fortunei*

The piecewiseSEM showed a positive effect of temperature on the density of the youngest larval stages of *L. fortunei*. This effect likely reflects the relationship between reproductive synchronism of *L. fortunei* and environmental seasonality. For instance, in South America, *L. fortunei* reproduction is continuous for 6–10 months of the year, but it reaches a peak in early summer when the temperature rises (Cataldo and Boltovskoy 2000; Boltovskoy 2015). The increased temperature is considered an important variable associated with the reproduction of *L. fortunei*, since accelerates the reproduction rate of the adults (Boltovskoy 2015). In addition, Cataldo et al. (2005) found that in temperatures around 28 ºC the larval development rate of *L. fortunei* also increases. Similarly, we found an average temperature of 29 ºC in December of 2011 and 2012 (Fig. S3a), and the density of the youngest larval stages reached a peak in these months and was low during the colder months (June and September; Fig. S2 and S4). Such findings illustrate a likely synchronism between reproduction and larval development of *L. fortunei* with increasing temperature in South American rivers (Cataldo et al. 2005; Boltovskoy 2015). A worrying factor is that, in its 5th report, the IPCC estimates that global temperatures will have increased by 1.2 °C to 4 °C by 2100 (IPCC 2013) and according to our results the temperature is a factor that can increase the reproduction rate de *L. fortunei*, and with the temperature rising proportionated by the climate change, this situation can be aggravated.

The water level had a direct negative effect on the density of the youngest larval stages of *L. fortunei*, which likely reflects a dilution and dispersion effect of the floods (Junk et al. 1989). As mentioned above, the density of the youngest larval stages reaches a peak in December during the beginning of the rainy season when water levels start to increase (Moi et al. 2020). The rising water level starts to disperse the larvae for the environments adjacent, such as lakes and ponds in January

(Amo et al. 2021). However, our sampling of *L. fortunei* is carried out in March when most of the larvae have already dispersed to adjacent environments. Therefore, when the water level reached the peak in March in rivers and channels (2011 = 7.2 m; 2012 = 6.34 m; Fig. S3b), the density of the youngest larval stages was low (see, Fig. S4) and the larvae remaining in these environments likely were diluted by the high-water level. A recent study conducted in the Upper Paraná River floodplain showed an increase in larval density of *L. fortunei* in march month in lakes adjacent to the studied rivers (Amo et al. 2021), which corroborates our findings, indicating that water level increases the spread of *L. fortunei* from these rivers that are propagule sources of this invasive mussel.

The piecewiseSEM also revealed that the water level exerted indirect positive effects on the density of the youngest larval stages of *L. fortunei*. Studies demonstrated that upper Paraná River floodplain, the high-water level during floods increases the water exchanges among rivers and adjacent environments, leading to dilution of the water, which reduces suspended matter and consequently the turbidity of these environments (Thomaz et al. 2004). This situation can provide a positive effect on the youngest larval *L. fortunei*. The high turbidity and suspended inorganic matter overload the filter system of the youngest larval stages, as individuals have small dimensions, which makes filtration difficult, leading to significant energy losses (e.g., Ernandes-Silva et al. 2016). In addition, high suspended inorganic matter reduces the quality of the suspended matter as food, both by decreasing the proportion of suspended organic material and by increasing energy expenditures in sorting out and eliminating the energetically unprofitable particles (Tokumon et al. 2015). Thus, the water level may favor *L. fortunei* establishment by spreading its larvae to adjacent environments (Amo et al. 2021) and by weakening local abiotic filters, such as turbidity and suspended inorganic matter.

Our results demonstrated also that there was a positive relationship between pH and density of the Umbonated larval. The low pH (i.e., < 6) limits the valve development and differentiation of *L. fortunei* (Checa et al. 2007), which occurs mainly during the Umbonated larval stage (Ezcurra de Drago et al. 2009), consequently, low pH should limit more strongly this larval stage in particular. Indeed, we found that Umbonated larval stages only was present in alkaline pH (i.e., above 6.5; see, Fig. S5).

2.5 Conclusion

In conclusion, our study demonstrated an expanding population pyramid of *L. fortunei* along the Upper Paraná River floodplain. We show that four of the five studied environments have a high larval density of *L. fortunei*, and the youngest larval stages are the most abundant in these

environments, mainly in December month when the temperature is higher than in other moths. The Ivinhema River is the only environment that has a low larval density of *L. fortunei*, and the youngest larval stages are less abundant in this environment. We also show that turbidity and the suspended inorganic matter have strong negative effects on the density of the youngest larval stages. Furthermore, turbidity and suspended inorganic matter are substantially higher in the Ivinhema River than in the other four environments. Most importantly, the Ivinhema River is also the only environment situated within a permanent preservation area (Braghin et al. 2018) and maintains high turbidity and suspended inorganic matter. Thus, our results evidence high turbidity and suspended inorganic matter are key factors reducing the density of the youngest larval stages of *L. fortunei*. Consequently, these two abiotic filters may be key to avoid the establishment of this nuisance invasive mussel. Our study also demonstrated that the youngest larval stages of *L. fortunei* appear to be most sensitive to abiotic filters while the oldest larval stages are more resistant. Thus, managing the youngest larval stages should be more advantageous to prevent the establishment of *L. fortunei*. Finally, *L. fortunei* is expected to invade other regions in the coming decades (Petsch et al. 2020). Thus, our results should be useful for understanding and controlling the spread and establishment of *L. fortunei* in other floodplains that have abiotic filters similar to those of the Upper Paraná River Floodplain.

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APPENDIX A - Additional analyzes of the age structure of the *L. fortunei* population and the effects of abiotic variables.

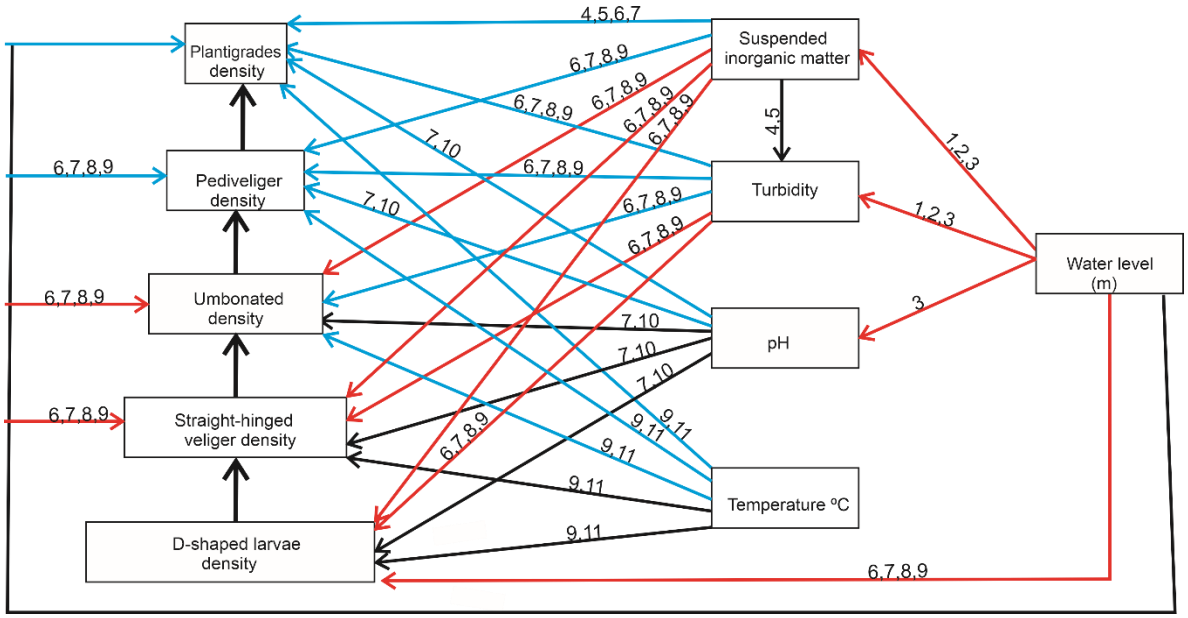

Figure S1. Theoretical predictive model representing the relationships among all abiotic filters and their consequent interactive effects on density of the larval stages of *L. fortunei*. Black and red arrows indicate a theoretical positive and negative relationship, respectively. Gray arrows denote a theoretical both relationships (positive and negative; i.e., studies have showed both positive and negative relationship between the two variables). To reduce the pollution of the graphs and make them easier to visualize, we only show the filters selected by Akaike's information criterion (AICc), that is, those filters excluded by AICc (total nitrogen, total phosphorus, nanoplankton biomass, and percentage of oxygen dissolved) are not shown in the graph. The numbers above the arrows represent the literature studies that supports the predicted relationships. For instance, the increase in water level often has a negative relationship with turbidity and suspended inorganic matter (Loverde-Oliveira et al. 2009 [1]; Moi et al. 2021 [2]; Thomaz et al. 2007 [3]). Likewise, the increase in water level also is negative related with pH (Thomaz et al. 2007 [3]). The increase in suspended inorganic matter increase turbidity (Thomaz et al. 2004 [4]; Mormul et al. 2012 [5]) In addition, abiotic filters differently affect the larval stages of *L. fortunei*. For instance, the increase in water level, turbidity, and suspended inorganic matter are negatively related with density of the two youngest larval stages in floodplain rivers (Tokumon et al. 2015 [6]; Ernandes-Silva et al. 2016 [7]; Amo et al. 2021 [8]; Boltovskoy 2015 [9]). pH also has a negative relationship with youngest larval stages of *L. fortunei*, because impair valve formation in these stages (Ezcurra de Drago et al. 2009 [10]; Ernandes-Silva et al. 2016 [7]). In addition, temperature is predicted to be positively related with density of the youngest larval stages because increase reproduction of adult stages (Boltovskoy

2015 [9]; Cataldo and Boltovskoy 2000 [11]). The biomass of the phytoplankton is expected to have a negative relationship with oldest larval stages (Ernandes-Silva et al. 2016 [7]).

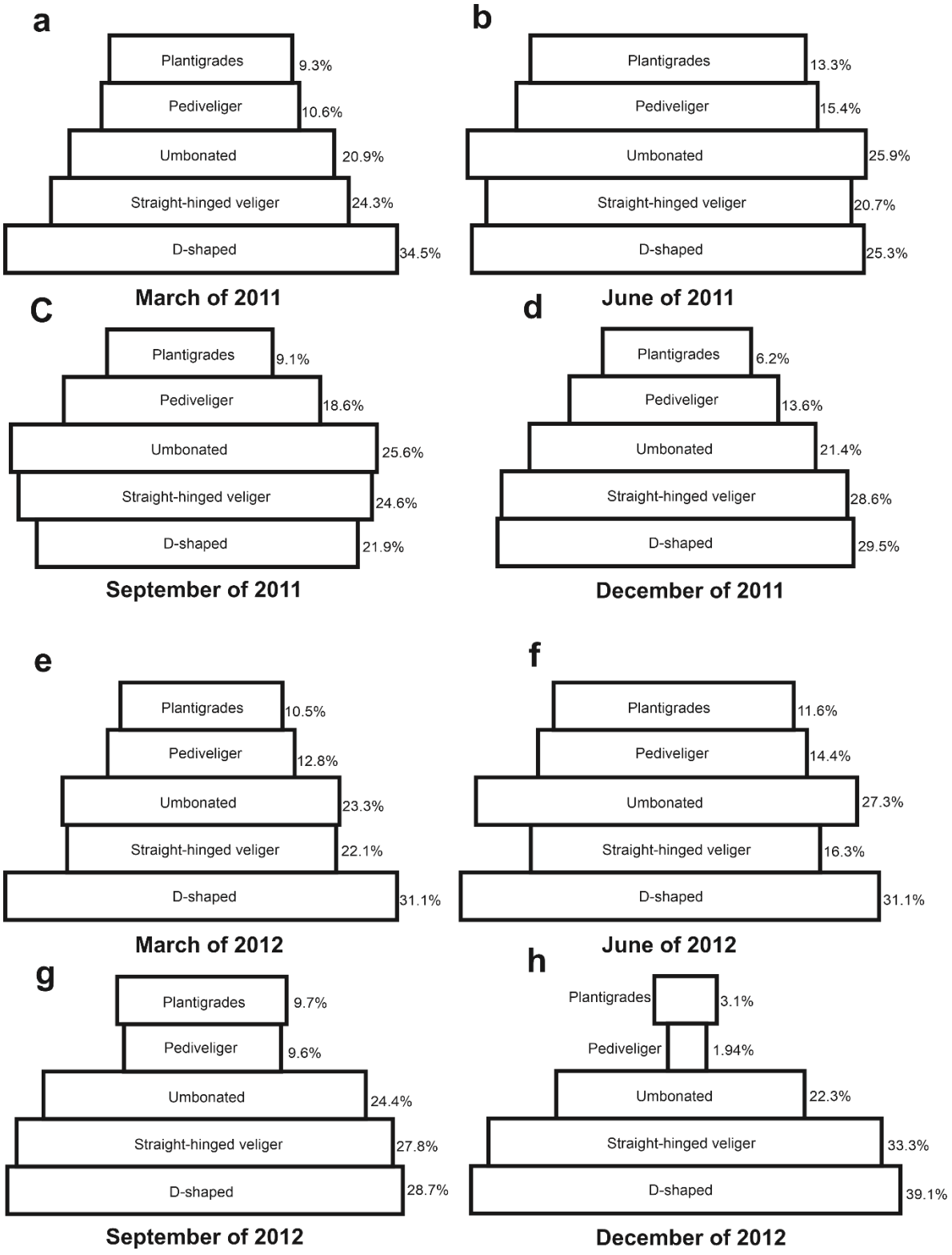

Figure S2. Population pyramid of *L. fortunei* across five studied environments. The values on the right represent mean percentage of each larval stages in each sample month.

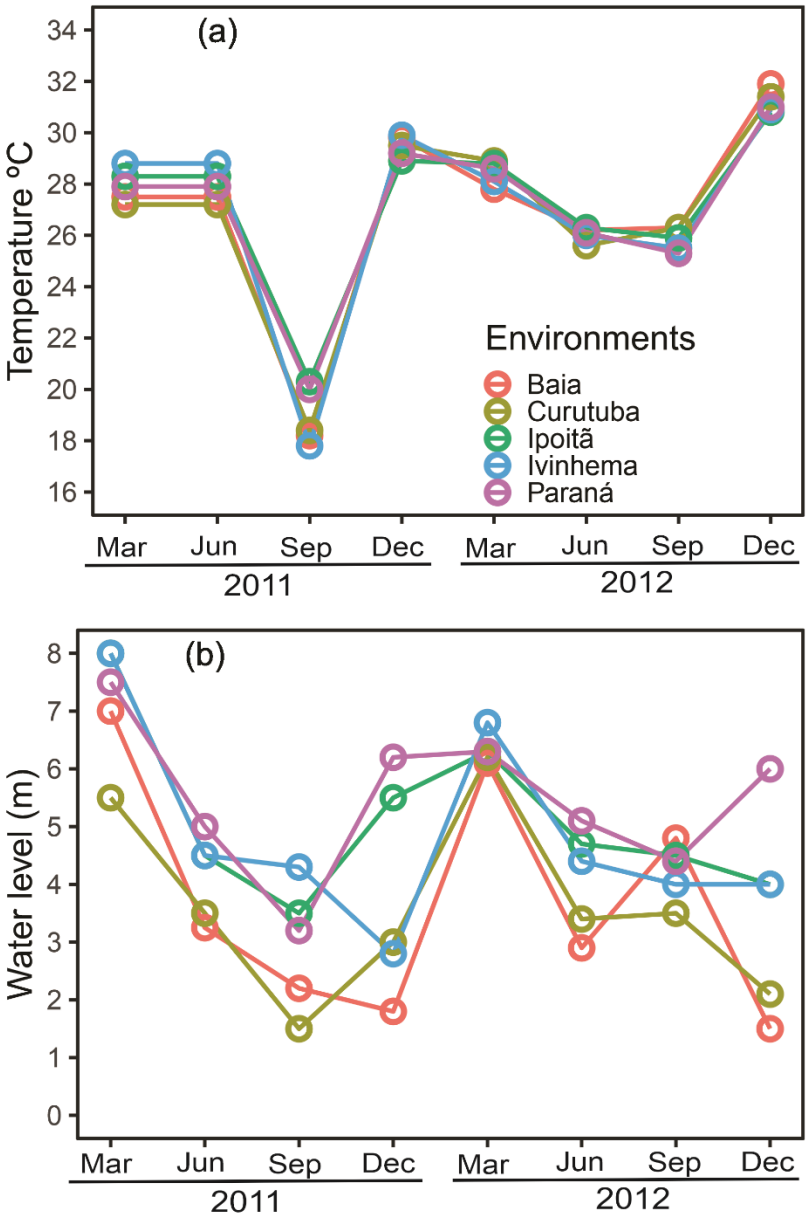

Figure S3. Values of (a) temperature and (b) water level in each of the five environments (Baía River, Curutuba channel, Ipoitã channel, Ivinhema River, and Paraná River) over each of the sampled periods (i.e., March, June, September, and December of 2011 and 2012).

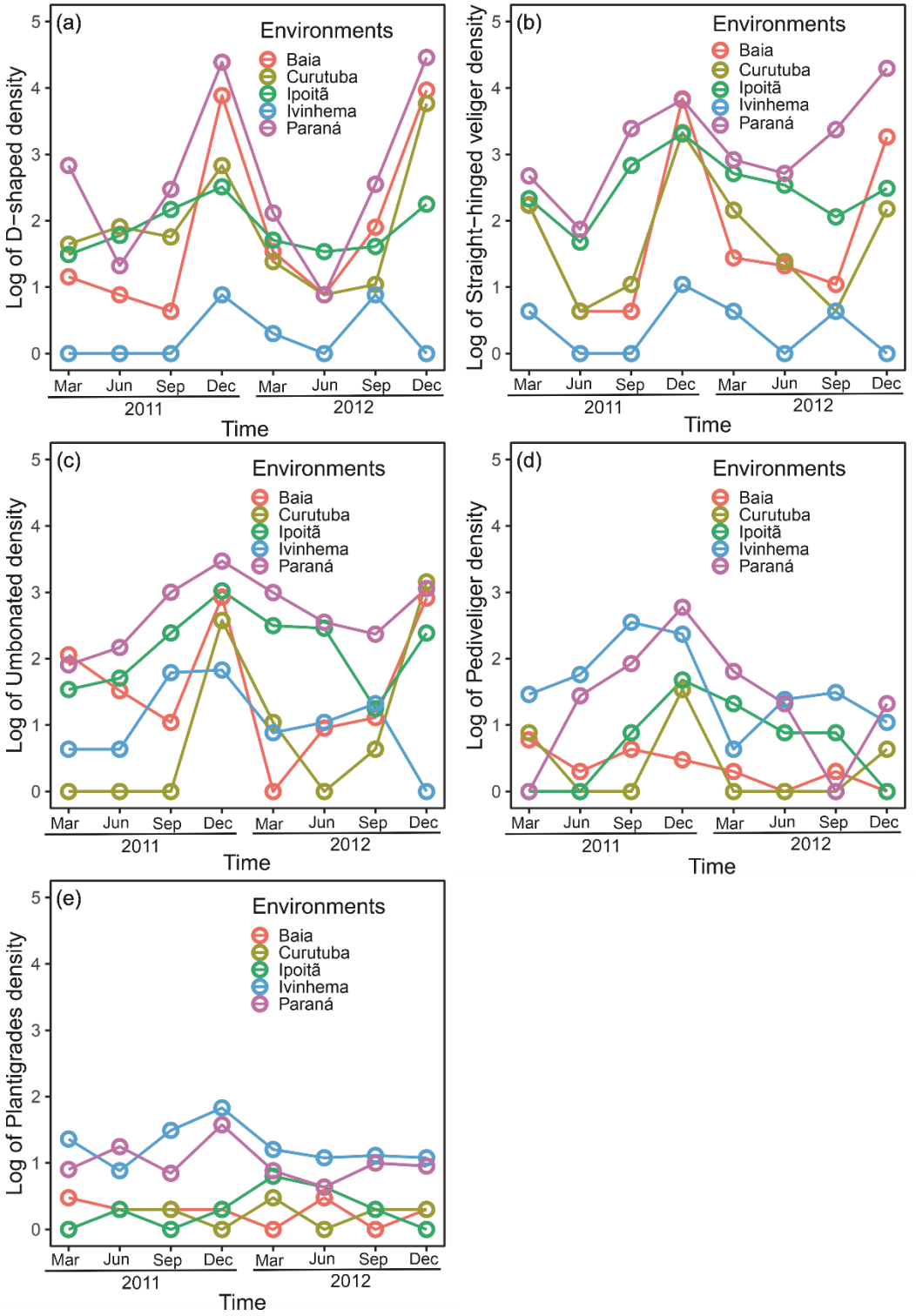

Figure S4. Density values of the larval stages of *L. fortunei* over studied period.

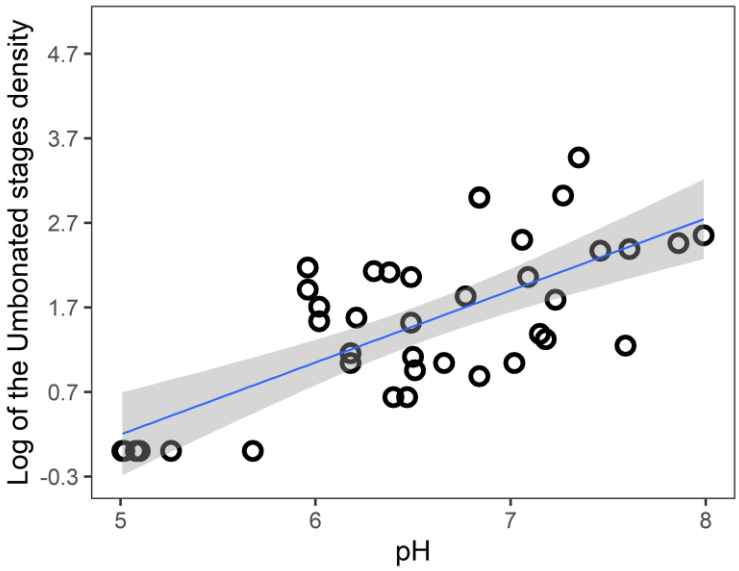

Figure S5. Relationship between pH and density of Umbonated larval stages.

Table S1. Pairwise comparisons of the density of the larval stages of *L. fortunei* among the five studies environments (Baía River, Paraná River, Ivinhema River, Curutuba channel, and Ipoitã channel. Pairwise was estimated by using Tukey´s HSD test in glht function of the 'multcomp' package (Hothorn et al. 2013).

Predictors	Estimate	*t*-value	*P*-value
D-shaped – First larval stage			
Environment comparison			
Curutuba vs Baía	0.045	0.122	0.999
Ipoitã vs Baía	0.150	0.399	0.994
Ivinhema vs Baía	–1.472	–3.917	0.003**
Parana vs Baía	0.521	1.387	0.639
Ipoitã vs Curutuba	0.104	0.277	0.998
Ivinhema vs Curutuba	–1.518	–4.038	0.002**
Parana vs Curutuba	0.475	1.265	0.713
Ivinhema vs Ipoitã	–1.623	–4.316	0.001
Parana vs Ipoitã	0.371	0.988	0.858
Parana vs Ivinhema	1.994	5.304	< 0.001***
Straight-hinged veliger – Second larval stage			
Environment comparison			
Curutuba vs Baía	–0.102	–0.259	0.998
Ipoitã vs Baía	0.690	1.741	0.423
Ivinhema vs Baía	–1.433	–3.614	0.007**
Parana vs Baía	1.080	2.723	0.070
Ipoitã vs Curutuba	0.793	1.999	0.287
Ivinhema vs Curutuba	–1.331	–3.355	0.015*
Parana vs Curutuba	1.182	2.981	0.0390*
Ivinhema vs Ipoitã	–2.124	–5.355	< 0.001***
Parana vs Ipoitã	0.389	0.982	0.8615
Parana vs Ivinhema	2.513	6.337	< 0.001***
Umbonated – Third larval stage			
Environment comparison			
Curutuba vs Baía	–0.789	–2.645	0.083
Ipoitã vs Baía	0.545	1.826	0.375
Ivinhema vs Baía	–0.467	–1.566	0.528
Parana vs Baía	1.080	3.619	0.007**
Ipoitã vs Curutuba	1.334	4.471	< 0.001***
Ivinhema vs Curutuba	0.321	1.079	0.816
Parana vs Curutuba	1.869	6.264	< 0.001***
Ivinhema vs Ipoitã	–1.012	–3.392	0.013*
Parana vs Ipoitã	0.535	1.793	0.393
Parana vs Ivinhema	1.547	5.185	< 0.001***
Pediveliger – Fourth larval stage			
Environment comparison			
Curutuba vs Baía	0.032	0.100	0.999
Ipoitã vs Baía	0.357	1.096	0.807
Ivinhema vs Baía	1.237	3.797	0.004**
Parana vs Baía	0.974	2.990	0.038*
Ipoitã vs Curutuba	0.324	0.996	0.855
Ivinhema vs Curutuba	1.205	3.696	0.006**
Parana vs Curutuba	0.942	2.890	0.048*

cont.

cont.

Predictors	Estimate	*t*-value	*P*-value
Ivinhema vs Ipoitã	0.880	2.701	0.074
Parana vs Ipoitã	0.617	1.894	0.339
Parana vs Ivinhema	–0.262	–0.806	0.926
Plantigrades – Fifth larval stage			
Environment comparison			
Curutuba vs Baía	–0.059	–0.465	0.990
Ipoitã vs Baía	0.022	0.179	1.000
Ivinhema vs Baía	0.985	7.691	< 0.001***
Parana vs Baía	0.736	5.743	< 0.001***
Ipoitã vs Curutuba	0.082	0.644	0.967
Ivinhema vs Curutuba	1.045	8.156	< 0.001***
Parana vs Curutuba	0.795	6.208	< 0.001***
Ivinhema vs Ipoitã	0.962	7.512	< 0.001***
Parana vs Ipoitã	0.713	5.564	< 0.001***
Parana vs Ivinhema	–0.249	–1.948	0.312

Table S2. Pairwise comparisons of the percentage among larval stages of *L. fortunei* within each of the five environments (Baía River, Paraná River, Ivinhema River, Curutuba channel, and Ipoitã channel. Pairwise was estimated by using Tukey´s HSD test in glht function of the 'multcomp' package (Hothorn et al. 2013).

Predictors	Estimate	*t*-value	*P*-value
Baía river			
Larval pairwise comparison			
Straight-hinged vs D-shaped	–6.421	–0.678	0.959
Umbonated vs D-shaped	–6.834	–3.888	0.003**
Pediveliger vs D-shaped	–6.430	–3.846	0.004**
Plantigrades vs D-shaped	–9.586	–4.179	0.001**
Umbonated vs Straight-hinged	–0.412	–3.210	0.022*
Pediveliger vs Straight-hinged	–0.009	–3.168	0.024*
Plantigrades vs Straight-hinged	–3.165	–3.501	0.010*
Pediveliger vs Umbonated	0.403	0.043	1.000
Plantigrades vs Umbonated	–2.752	–0.291	0.858
Plantigrades vs Pediveliger	–3.156	–0.333	0.997
Curutuba channel			
Larval pairwise comparison			
Straight-hinged vs D-shaped	8.806	2.771	0.063
Umbonated vs D-shaped	–2.612	–2.175	0.212
Pediveliger vs D-shaped	–6.358	–2.536	0.105
Plantigrades vs D-shaped	–6.916	–2.589	0.094
Umbonated vs Straight-hinged	–1.419	–4.947	< 0.001***
Pediveliger vs Straight-hinged	–5.164	–5.307	< 0.001***
Plantigrades vs Straight-hinged	–5.722	–5.361	< 0.001***
Pediveliger vs Umbonated	–3.745	–0.360	0.996
Plantigrades vs Umbonated	–4.303	–0.414	0.993
Plantigrades vs Pediveliger	–0.557	–0.054	1.000
Ipoitã channel			
Larval pairwise comparison			
Straight-hinged vs D-shaped	9.059	9.763	< 0.001***
Umbonated vs D-shaped	4.610	2.908	0.046*
Pediveliger vs D-shaped	–0.488	–2.087	0.248
Plantigrades vs D-shaped	–1.231	–2.235	0.190
Umbonated vs Straight-hinged	–4.448	–6.855	< 0.001***
Pediveliger vs Straight-hinged	–9.547	–11.850	< 0.001***
Plantigrades vs Straight-hinged	–0.290	–11.998	< 0.001***
Pediveliger vs Umbonated	–5.099	–4.995	< 0.001***
Plantigrades vs Umbonated	–5.841	–5.143	< 0.001***
Plantigrades vs Pediveliger	–0.742	–0.148	< 0.001***
Ivinhema river			
Larval pairwise comparison			
Straight-hinged vs D-shaped	-0.032	-0.004	1.000
Umbonated vs D-shaped	5.523	0.664	0.962
Pediveliger vs D-shaped	6.104	4.343	< 0.001***
Plantigrades vs D-shaped	7.518	2.107	0.239
Umbonated vs Straight-hinged	5.556	0.668	0.961
Pediveliger vs Straight-hinged	6.137	4.347	< 0.001***
Plantigrades vs Straight-hinged	7.550	2.111	0.238

cont.

cont.

Predictors	Estimate	*t*-value	*P*-value
Pediveliger vs Umbonated	0.581	3.678	0.006**
Plantigrades vs Umbonated	1.994	1.443	0.605
Plantigrades vs Pediveliger	–8.586	-2.236	0.190
Paraná river			
Larval pairwise comparison			
Straight-hinged vs D-shaped	0.509	2.803	0.058
Umbonated vs D-shaped	2.868	0.392	0.994
Pediveliger vs D-shaped	–4.905	–3.404	0.013*
Plantigrades vs D-shaped	–6.195	–3.581	0.008**
Umbonated vs Straight-hinged	–7.640	–2.411	0.136
Pediveliger vs Straight-hinged	–5.414	–6.208	< 0.001***
Plantigrades vs Straight-hinged	–6.704	–6.384	< 0.001***
Pediveliger vs Umbonated	–7.773	–3.796	0.004**
Plantigrades vs Umbonated	–9.063	–3.973	0.002**
Plantigrades vs Pediveliger	–1.290	–0.176	0.999

Table S3. Model selection of backward elimination by corrected Akaike information criterion (AICc) performed to predicts the most parsimonious predictors that influence larval stage of *L. fortunei* across environments.

Model selection steps	Variables removed from the full model	AICc	ΔAIC	Fishers´s C	*p*
L. fortunei model					
Full model	-	190.77	-190.77	32.77	0.109
1	TP	173.31	-198.07	25.31	0.191
2	TP + TN	158.45	211.26	20.45	0.201
3	TP + TN + Phyto	131.58	-263.16	13.58	0.093
Final model	TP + TN + Phyto + DO	120.81	-396.34	24.81	0.306

We included all steps of the model selection and the set of variables removed from the full model in each step. To evaluated model fit, we used Fisher´s C statistic and its associated *p*-value. Turb = Turbidity; TN = total nitrogen; TP = total phosphorus; Temp = temperature; Phyto = phytoplankton; SIM = suspended inorganic matter; DO = percent of dissolved oxygen; WL = water level; the full model to *L. fortunei* larval stages, with Y = density of the *L. fortunei* larval stage: Y ~ Turb+TN+TP+Temp+Phyto+SIM+DO+pH+WL

Table S4. Results of structural equation modelling, fitted to larval golden mussel stages. The structural equation model was used to test direct and indirect effects of environmental predictors on five larval golden mussel stages. *= $P < 0.05$, **= $P < 0.01$ and ***= $P < 0.001$. SIM = suspended inorganic matter

Models			**Std.error**	**DF**	**Standardized path coefficients**	***P*-value**
MEP – full model						
Response		Predictor				
SIM	<--	water level	0.0123	28	−0.279	**0.002****
Turbidity	<--	water level	0.1012	28	−0.509	**< 0.001*****
Turbidity	<--	SIM	0.0041	28	0.720	**< 0.001*****
Plantigrades	<-->	Pediveliger			0.827	**< 0.001*****
Plantigrades	<--	SIM	0.4547	28	−0.116	0.213
Plantigrades	<--	pH	0.1425	28	0.061	0.638
Plantigrades	<--	Turbidity	0.2006	28	0.032	0.786
Plantigrades	<--	Temperature	0.0226	28	0.106	0.356
Plantigrades	<--	water level	0.0657	28	0.067	0.365
Pediveliger	<-->	Umbonated			0.694	**< 0.001*****
Pediveliger	<--	SIM	0.2020	28	0.124	0.379
Pediveliger	<--	pH	0.1966	28	0.041	0.786
Pediveliger	<--	Turbidity	0.3267	28	−0.020	0.904
Pediveliger	<--	Temperature	0.0278	28	−0.126	0.295
Pediveliger	<--	Hydrometric level	0.0891	28	0.027	0.848
Umbonated	<-->	Straight-hinged veliger			0.696	**< 0.001*****
Umbonated	<--	SIM	0.2244	28	0.192	0.082
Umbonated	<--	pH	0.2036	28	0.298	**0.012***
Umbonated	<--	Turbidity	0.4259	28	0.099	0.526
Umbonated	<--	Temperature	0.0355	28	−0.090	0.418
Umbonated	<--	water level	0.1020	28	0.149	0.203
Straight-hinged veliger	<-->	D-shaped larvae			0.708	**< 0.001*****
Straight-hinged veliger	<--	SIM	0.0998	28	−0.316	**0.003****
Straight-hinged veliger	<--	pH	0.1852	28	0.071	0.446
Straight-hinged veliger	<--	Turbidity	0.3786	28	−0.416	**0.0186***
Straight-hinged veliger	<--	Temperature	0.0334	28	−0.049	0.600
Straight-hinged veliger	<--	water level	0.0999	28	0.122	0.287
D-shaped larvae	<--	SIM	0.0103	28	−0.510	**< 0.001*****
D-shaped larvae	<--	pH	0.2443	28	0.061	0.625
D-shaped larvae	<--	Turbidity	0.4120	28	−0.713	**< 0.001*****
D-shaped larvae	<--	Temperature	0.0377	28	0.455	**0.001****
D-shaped larvae	<--	water level	0.1196	28	−0.686	**0.008****

Table S5. Average and standard deviation of the limnological variables in each sampled habitat. DO = dissolved oxygen, SIM = suspend inorganic matter, TN = total nitrogen, TP = total phosphorus

Variables	Paraná R.	Ipoitã C.	Ivinhema R.	Curutuba C.	Baía R.
Temperature (°C)	26.8±3.2	27±3.1	26.8±4	26.6±3.7	26.6±3.83
DO (%)	92.28±13.6	86.67±18.1	79.53±19.08	69.37±10.8	78.33±9.95
pH	6.9±0.7	7.1±0.7	6.8±0.3	6.1±0.3	6.4±0.3
Turbidity (NTU)	1.7±1.9	4.1±3	28.2±11.9	8.0±4.1	2.5±3.6
SIM	0.19±0.4	1.35±0.8	7.02±0.6	0.66±0.2	0.21±0.2
TN	706.0+94.0	719.6±130.7	956.6±343.1	810.2±80.3	729.3±153.8
TP	10.91±7.5	11.95±4.8	44.96±21.5	34.17±15.8	30.80±12.2
Hydrometric level (m)	5.57±1.4	4.62±1.4	4.75±1.3	3.06±0.9	3.24±1.5

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3 IMPACTS OF *Limnoperna fortunei* (DUNKER, 1857) ON TAXONOMIC AND FUNCTIONAL DIVERSITY OF PHYTOPLANKTON COMMUNITY

ABSTRACT

Limnoperna fortunei is one of the most widespread invaders in aquatic ecosystems worldwide, and it has been linked to impacts on the phytoplankton community, which could have negative consequences to the primary productivity of aquatic ecosystems. However, this relationship has not yet been studied in detail. Here, we used a three-year database from five freshwater ecosystems to investigated how the increased density of *L. fortunei* (larval stages) affect taxonomic and functional diversity of phytoplankton communities, and its consequences to biomass stock of the phytoplankton. We found that increases in density of *L. fortunei* was strongly related to decreased in taxonomic richness and functional diversity of the phytoplankton communities. The increase in density of *L. fortunei* was associated to decrease in the phytoplankton biomass stock through decreased in taxonomic and functional diversity of phytoplankton. Our study illustrates that *L. fortunei* has the potential to decrease both taxonomic and functional diversity of the phytoplankton community, and may negatively affect the primary production of invaded aquatic ecosystems.

Keywords: biological invasion, ecosystem functioning, golden mussel, functional traits, primary production.

3.1 Introduction

Biological invasions are among the major global threats to biodiversity and ecosystem functioning (Simberloff et al., 2013; Linders et al., 2019). Invasive species cause strong ecological and economic pressure on marine, terrestrial and freshwater ecosystems (Simberloff et al., 2013; Gallardo et al., 2016). Given the growing number of new invasive species across the globe (Seebens et al., 2017), investigating how multiple facets of the native biodiversity and functioning of invaded ecosystems respond to the increase in density of invasive species represents a vital challenge to the management and conservation of natural ecosystems. Previous studies have found that as invasive species increase in density, a continuous loss of taxonomic richness of native species is observed (Rahel, 2002; Dar & Reshi, 2020). However, it is still unclear how other native biodiversity facets, such as functional diversity, respond to the increased density of invasive species. We also know relatively little about how the impacts of invasive species on multiple facets of the native biodiversity affect the functioning of invaded ecosystems (Havel et al., 2015).

Aquatic ecosystems are especially sensitive to introducing invasive species, since these ecosystems suffer from anthropogenic impacts, such as eutrophication (Picart et al., 2015) and construction of reservoirs (Couto & Olden, 2018). In addition, aquatic ecosystems such as floodplain systems present high connectivity, which favors the dispersion of invasive propagules (Meghan et al., 2018; Amo et al., 2021). These factors facilitate the introduction of invasive species with high dispersion ability and capacity to modify ecosystem structure (Sala et al., 2000; Moi et al., 2021b). A critical example of invasive species invading and modifying aquatic ecosystems are freshwater mussels, such as *Dreissena polymorpha*, a widespread invader in the northern hemisphere, and the *Limnoperna fortunei* (Dunker, 1857), popularly known as golden mussel, which invaded large areas of Asia and South America (Boltovskoy et al., 2006; Boltovskoy & Correa, 2015; Petsch et al., 2020). *L. fortunei* presents short life cycle, high fertility, high growth rate (Damborenea & Penchaszadeh, 2006) and two shells made of calcium carbonate, which provide an ecological advantage in protecting them from external factors, such as temperature variation, salinity, pH and predation (Darrigran, 2002; Uliano-Silva et al., 2016). *L. fortunei* has a high dispersal capacity, as it has a planktonic larval stage and an encrusting adult. These different forms throughout its life cycle facilitate its dispersion between environments, since the larval stage of *L. fortunei* is easily dispersed by running water and ballast water from commercial ships (González-Bergonzoni et al., 2020), while the adult stage is dispersed by vessel traffic colonized (Boltovskoy et al., 2006).

L. fortunei is considered a fouling pest that clogs industrial and water supply infrastructures, causing economic losses (Boltovskoy & Correa, 2015). Furthermore, the adults of *L. fortunei* is an

ecosystem engineer; thus, as its density increases, a series of ecology impacts are observed in invaded ecosystems, such as decreasing particulate organic matter, increasing nutrient concentration in the water column (Boltovskoy et al., 2015; Cataldo et al., 2012b) and increasing water transparency (Boltovskoy et al., 2015). Due high filtration rate of *L. fortunei*, their impacts are extreme on phytoplankton communities (Boltovskoy et al., 2009). Experimental studies showed that *L. fortunei* adults markedly decreased the richness and abundance of phytoplankton (Cataldo et al., 2012a; De Stefano et al., 2018). Knowledge about the impacts of *L. fortunei* on the functional diversity of phytoplankton communities is limited, although experimental evidence suggests that L. fortunei may select some specific phytoplankton characteristics, for example, preying on small algae and favoring toxic cyanobacteria (Cataldo et al., 2012a; Alcísio & Giani, 2018). Furthermore, if *L. fortunei* decreases taxonomic and functional diversity of phytoplankton communities, this should impair primary productivity of freshwater ecosystems (Boltovskoy & Correa, 2015), since phytoplankton is one the main primary producer of these ecosystems (Field et al., 1998). However, these effects have not been explored in natural ecosystems, nor for the larval stages.

L. fortunei larvae are already able to feed actively from their first stage (larva D, Ezcurra de Drago et al., 2009), when their veil is completely formed, and despite being small (90 µm - 490 µm), they can reach densities quite high (around 2,000 ind. m^{-3}, Oliveira et al., 2011), and thus promote impacts on the resource community (phytoplankton).

L. fortunei is expected to successfully occur in several regions worldwide in the next few decades (Petsch et al., 2020). This is extremely worrisome because the increasing spread of *L. fortunei* have impaired the food web and functioning of highly diverse freshwater ecosystems (Boltovskoy & Correa, 2015; González-Bergonzoni et al., 2020). Therefore, in order to understanding environmental impacts of *L. fortunei* it has become urgent to investigate how *L. fortunei* affects multiple facets of biodiversity and the functioning of invaded ecosystems. In this study, we used three-year database from five freshwater ecosystems to investigate the relationship of *L. fortunei* (larval stages) with the taxonomic and functional diversity of phytoplankton communities. Moreover, we also employed a structural equation modelling to investigate how *L. fortunei* affect the biomass stock of these ecosystems mediated by their effects on the taxonomic and functional diversity of the phytoplankton. We predict that as the density of *L. fortunei* increases, the taxonomic and functional diversity of phytoplankton decreases. We expect that the density of *L. fortunei* larvae show a negative relationship with the simpler functional traits, as these can be more easily preyed. Furthermore, as the taxonomic and functional diversity of phytoplankton decreases, we also predicted that the biomss stock of ecosystems would be negatively affected.

3.2 Materials and Methods

3.2.1 Study area

This study was carried out in a 230 km stretch in the upper Paraná River Floodplain (Fig. 1), which is free from damming (Agostinho et al., 2008). This region is highly diverse and considered an "Extreme Biological Importance" area. Because of their contributions to the maintenance of several species, the Protected Area of islands and floodplains of the Paraná River in all its extension were established in this location, and the Ilha Grande National Park and the Ivinhema Islands State Park, which contemplate the most habitats (Agostinho et al., 2013).

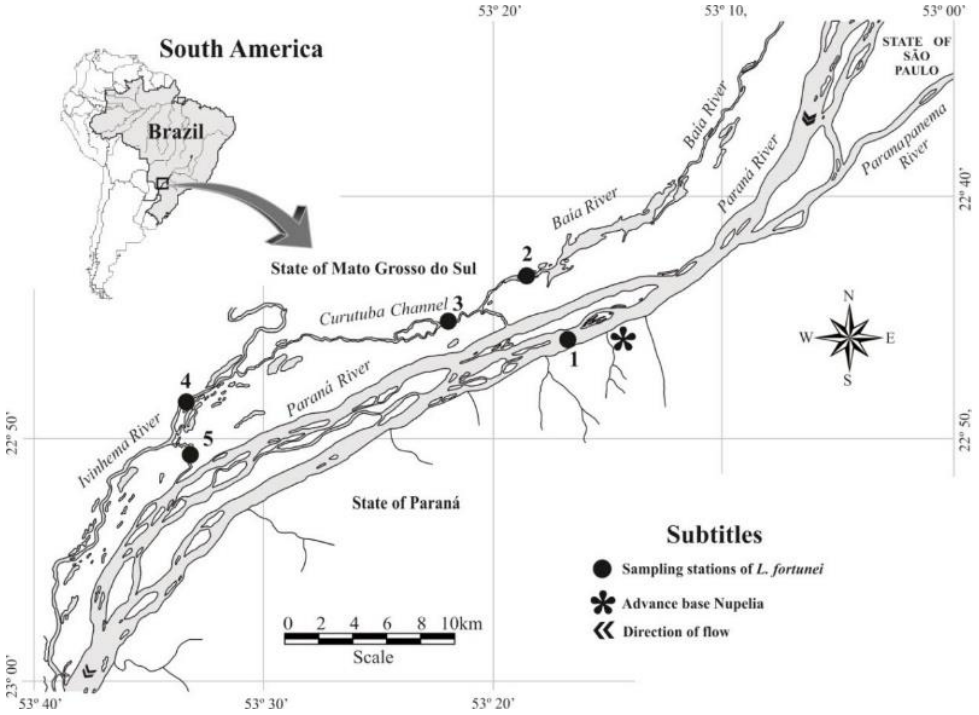

Fig. 1 Study area map with sampling stations. 1: Paraná River, 2: Baia River, 3: Curutuba Channel, 4: Ivinhema River, 5: Ipoitã Channel.

3.2.2 Samplings of golden mussel larvae

L. fortunei were collected quarterly during years of 2011 - 2013 (summer, spring, autumn, and winter). Sampling was took in the central region of five lotic environments of the Upper Paraná River Floodplain (Paraná River - 22º43'7" S; 53º13'4" W, Ipoitã channel - 22º50'08" S; 53º33'6" W, Ivinhema River - 22º51'23" S; 53º36'23" W, Baia River – 22º41'9" S; 53º15'8" W and, Curutuba channel - 22º45'2" S; 53º21'32" W; Fig 1) totaling 60 samples in time and space (3 years x 4 seasons x 5 lotic environments = 60 samples) For this, a plankton net (30 µm mesh opening) was used for the filtration of 300 L of water. The material was fixed in 80% alcohol and analyzed in the laboratory, with the aid of an optical microscope. The larvae were counted and their densities expressed in individuals m^{-3}. For samples with high larval density, aliquots (10 ml) were performed

using a Hensen-Stempell pipette. Of the 60 sampled points, this procedure was performed in only five of them.

3.2.3 Samplings of phytoplankton community

Simultaneously to *L. fortunei*, the phytoplankton community was sampled at the sub-surface in the pelagic zone using bottles and preserved in 10% acetic acid (Bicudo & Menezes, 2006). Phytoplankton density was estimated using an inverted microscope according to the American Public Health Association-APHA (1985), and the results are expressed in individuals (cells, cenobes, colonies or filaments) per millimeter (Uthermöhl, 1958). We analyzed phytoplankton structure using cell sizes: nanoplankton (< 60 µm) and microplankton (60-500 µm).

3.2.4 Phytoplankton taxonomic and functional diversity

In each sampling period, the phytoplankton was identified at the lowest possible taxonomic level (species) according to the classification system of Van Den Hoeck et al. (1995) for eukaryotic phytoplankton species, and the Anagnostidis & Komarék (1985, 1988) and Komárek & Anagnostidis (1986, 1989) for the Cyanobacteria species. Phytoplankton taxonomic diversity was then estimated as the number of phytoplankton species in each sampling period in each of five ecosystems. We classified phytoplankton functional traits according to specialized literature (e.g., Kruk et al., 2017; Ramond et al., 2019; Graco-Roza et al., 2021). Specifically, we used six functional traits combinations: body size (continuous; μm^3), mucilage (dummy), tendency to form colonies and chains (categorical: colonial; chains, nor form colonies or chains), motility (categorical: floating, gliding, swimming), flagellate (dummy), and siliceous walls (dummy; Kruk et al., 2017; Ramond et al., 2019; Graco-Roza et al., 2021). We used these six functional traits of the phytoplankton because it best reflects the effect of *L. fortunei* on phytoplankton community (see Table 1; *Supplementar material*).

3.2.5 Data analyses

Functional diversity of the phytoplankton was calculated using Rao's quadratic entropy (RaoQ), a common measure to estimate functional diversity (Botta-Dukát, 2005). RaoQ incorporates the weighted relative abundance of each species and converts it to effective numbers. Rao's Q calculate the variation of the distance among species based on Gower’s dissimilarity (Botta-Dukát, 2005). Trait matrix of phytoplankton had mixed variables (continuous and

categorical); thus, we used the Gower's dissimilarity with Cailliez's correction (Laliberté & Legendre, 2010). We also calculated community-weighted means (CWMs) for each functional trait (which was weighted by species' relative abundance) further to characterize the functional composition of the phytoplankton communities. We calculated the Rao's quadratic entropy (Rao's Q) and community-weighted means (CWMs) using the FD package (Laliberté et al., 2015) in R statistical software.

We evaluated separately the relationship of the log density of *L. fortunei* with (i) taxonomic richness, (ii) functional diversity (Rao's quadratic entropy), and (iii) CWM of each functional trait of the phytoplankton applying generalized linear mixed-effects models (GLMMs) using the lme4 package (Bates et al., 2014) in R statistical software (R Core Team, 2018). To account for potential non-independence of seasons, and to account for the effect of environment identity, we nested the seasons within year in each of the five environments were considered as a random structure. Thus, we allowed the intercept to vary in each season within the year independently for each environment. Our data met the assumptions of the Poisson distribution; thus, we used a GLMM with a Poisson distribution. As our data had a time series, samplings closer in time are likely to be more similar than those farther apart. To correct this potential temporal bias, we addressed any potential temporal autocorrelation by modelling a correlation among sampling periods using a continuous autoregressive 1 autocorrelation structure from the CAR1 function.

We use structural equation modelling (pSEM; Lefcheck, 2016) to assess the direct and indirect effects of *L. fortunei* on the functional and taxonomic diversity of phytoplankton, and on ecosystem functioning (primary productivity). The water level was explicitly included as an exogenous variable in the model because it is a crucial environmental driver affecting *L. fortunei* and the functioning of aquatic environments (Amo et al., 2021; Moi et al., 2021a). We also included in the model the water transparency and N:P ratio, which are two important environmental factors influenced by *L. fortunei* (Boltovskoy et al., 2015, Cataldo et al., 2012b), and change in these two factors may affect taxonomic and functional diversity of the phytoplankton with consequences to their biomass. Water transparency was measured by using Secchi depth, and N:P were measured using total factions of phosphorus and nitrogen. Total Nitrogen (N) was analyzed by a persulphate method (Bergamin et al., 1978) and determined in a spectrophotometer in the presence of cadmium, using a flow-injection system (Giné et al., 1980). Total phosphorus (P) was measured according to (Golterman et al., 1978). We nested the seasons within year in each of the five environments as a random structure. Potential temporal autocorrelation in piecewiseSEM was addressed by modelling a correlation among sampling periods using a continuous autoregressive 1 autocorrelation structure from the CAR1 function. We tested multicollinearity for each model component by calculating the

variance inflation factor (VIF). VIF > 3 indicates possible collinearity, which was not observed in our model. The piecewiseSEM was fitted using a linear mixed-effect model in the piecewiseSEM package (Lefcheck, 2016). We presented the standardized coefficient for each path and estimated the indirect effects by coefficient multiplication. Path significance was obtained by maximum likelihood and model fit was evaluated using Shipley's test of d-separation through Fisher's C statistic ($P > 0.05$ indicates adequate model).

3.3 Results

Over three-years sampling, we found 95,474 larvae of *L. fortune,* and 14,634 organisms of phytoplankton, distributed in 224 species. Overall, the increase in density of *L. fortunei* was negatively related to taxonomic and functional diversity of the phytoplankton (Table 1). Among the facets of phytoplankton diversity, the functional was the most strongly affected by the increase in the density of golden mussel larvae (Table 1). Thus, as increased density of *L. fortunei*, the taxonomic and functional diversity of the phytoplankton decreased (Fig. 2). In addition, the increased in density of *L. fortunei* was associated with decreased in abundance of small-sized phytoplankton that lacked silica wall, flagella, mucilage, that did not form colonies or chains, and that lacked motility (Table 1, Fig. 3). This was evidenced because, as the increased density of *L. fortunei*, the CWM of body size, presence of siliceous walls, flagellum presence, form colonies and chains, and swimming motility increased (Fig. 3). Conversely, as increased density of *L. fortunei*, the CWM of the absence of siliceous walls, flagellum absence, mucilage absence, no form colonies and chains, and floating motility decreased (Fig. 3).

Table 1. Relationship of log the density of *L. fortunei* with taxonomic and functional diversity of the phytoplankton, and CWM of each individual traits of the phytoplankton. Detailed outcomes of the generalized linear mixed-effect models. CI = 95% confidence interval, Edf = degrees of freedom, Std.Error = standard error of the estimate. $R^2_{marginal}$ was estimated by *r.squaredGLMM* function in package MuMIn.

Dependent variables	Log of abundance of *L. fortunei*					
	Estimate	Std.Error	Edf	t-value	p-value	$R^2_{marginal}$
Taxonomic richness	-0.021	0.000	41	-2.065	**0.045**	0.254
Functional diversity-FD	-0.317	0.048	41	-6.488	< **0.001**	0.406
CWM of body size	0.275	0.067	41	4.072	< **0.001**	0.263
CWM of sicileous walls presence	0.035	0.015	41	2.237	**0.030**	0.085
CWM of sicileous walls absence	-0.273	0.059	41	-4.629	< **0.001**	0.259
CWM of flagellum presence	0.032	0.028	41	1.128	0.265	0.025
CWM of flagellum absente	-0.120	0.053	41	-2.244	**0.030**	0.085

cont.

cont.

Dependent variables	Log of abundance of *L. fortunei*					
	Estimate	Std.Error	Edf	t-value	p-value	$R^2_{marginal}$
CWM of mucilage presence	-0.170	0.116	41	-1.469	0.149	0.038
CWM of mucilage absence	-0.094	0.022	41	-4.183	< **0.001**	0.242
CWM of form colonies	0.466	0.090	41	5.164	< **0.001**	0.346
CWM of form chains	0.031	0.015	41	2.070	**0.044**	0.079
CWM of no form colonies or chains	-0.514	0.067	41	-7.644	< **0.001**	0.471
CWM of floating motility	-0.204	0.054	41	-3.756	< **0.001**	0.203
CWM of gliding motility	-0.143	0.055	41	-2.592	**0.013**	0.096
CWM of swimming motility	0.098	0.024	41	4.100	< **0.001**	0.251

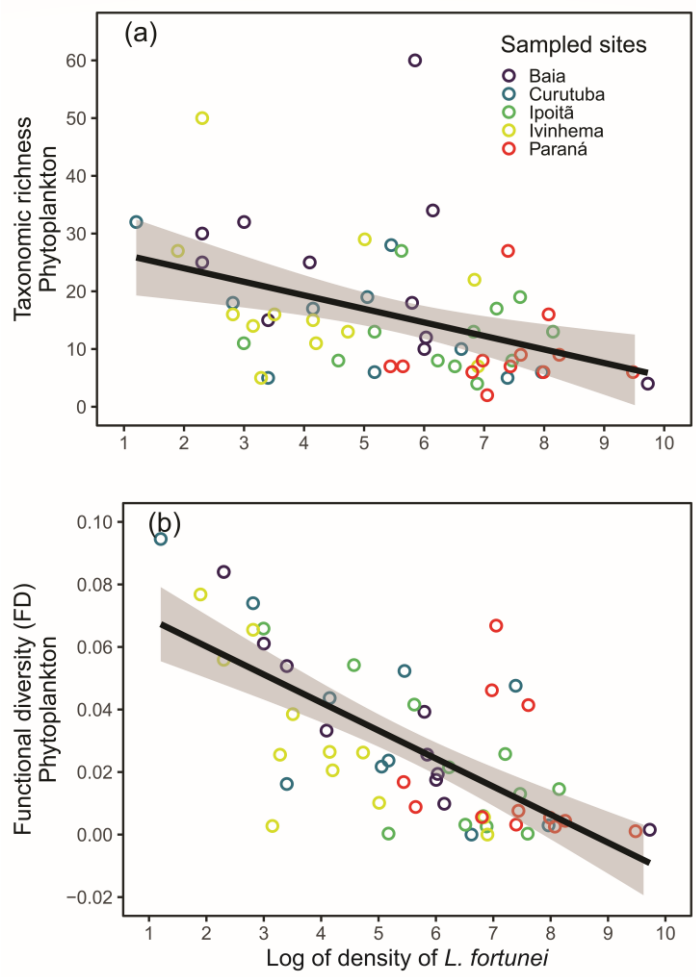

Fig. 2 Plotted values are the partial effects of the density of *L. fortunei* on the (a) taxonomic richness and (b) functional diversity of the phytoplankton across different environments in the upper Paraná River floodplain. Points with different colors indicate different locations. Fitted lines are generalized linear mixed regressions ± 95% confidence intervals.

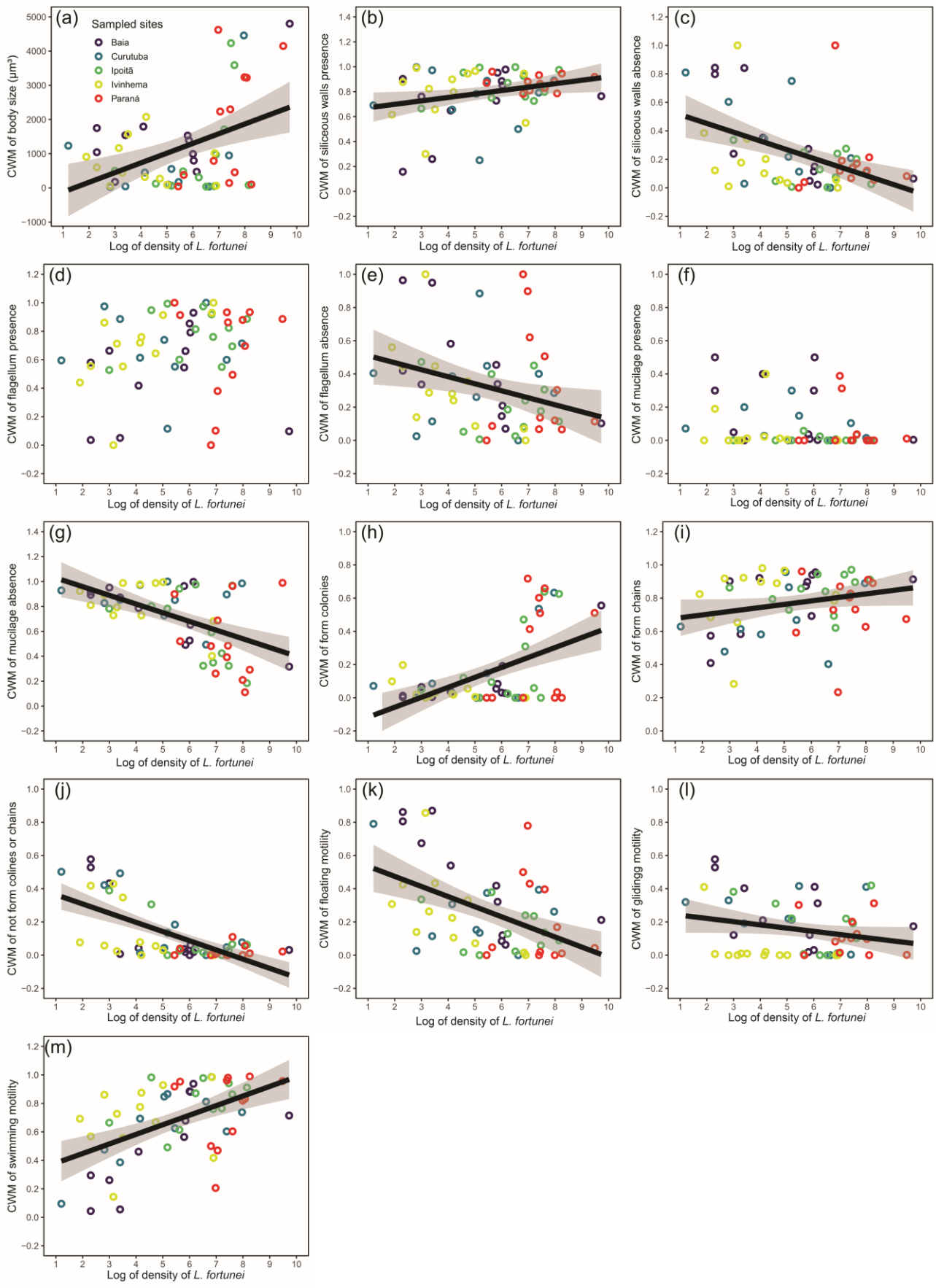

Fig. 3 Relationship of log the density of *L. fortunei* with CWM of each individual traits of the phytoplankton.

The piecewiseSEM fitted our data well (AICc = 278.25, Fisher`s C = 4.989, P = 0.958), and showed direct and indirect negative relationships between *L. fortunei* density with taxonomic and functional diversity of the phytoplankton, which also had negative consequences to their biomass stock. Specifically, *L. fortunei* density directly decreased the taxonomic and functional diversity of the phytoplankton (Fig. 4). *L. fortunei* also indirectly decreased the phytoplankton taxonomic (r = -0.185) richness by decreasing N:P ratio (Fig. 4). Although water level and *L. fortunei* density have increased water clarity, this did not affect the phytoplankton (Fig. 4). Finally, the negative relationships of *L. fortunei* with taxonomic and functional diversity of the phytoplankton decreased the biomass stock of the phytoplankton (taxonomic: r = -0.110; functional: r = -0.381; Fig. 4).

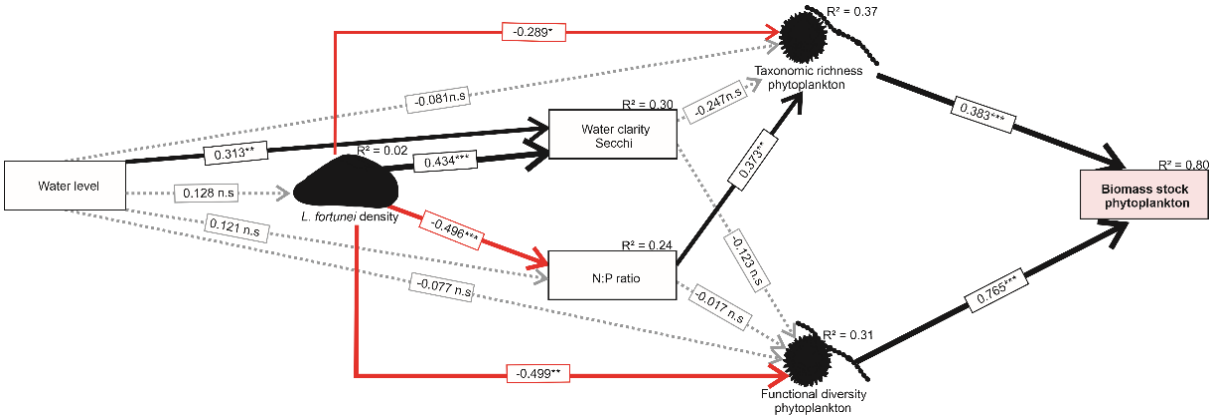

Fig. 4 Structural equation models of the relationship between density of larvae of *L. fortunei* and functional and taxonomic diversity and biomass of phytoplankton community. Solid black and red arrows represent significant positive and negative paths, respectively. R^2 for component models are given above the boxes of endogenous variables. The non-significant patches results were dashed line. N.S**:** non significative relationship, and *P<0.05, ** P<0.01,***P<0.001.

3.4 Discussion

Our results revealed that the increased density of *L. fortunei* was related to decreased taxonomic richness of the phytoplankton community. These results are similar to those obtained with other invasive mussels, such as *Dreissena polymorpha* (Pallas) (Feniova et al., 2020) and *Corbicula fluminea* (Müller, 1774) (Minaudo et al., 2021), suggesting that invasive mollusks cause loss of taxonomic diversity of phytoplankton communities. Most importantly, we showed that *L. fortunei* was related to a markedly decrease in functional diversity of the phytoplankton, what can be an indication of homogenization taxonomically and functionally the phytoplankton. In addition, there was a substantial decreased in algal biomass stock as phytoplankton taxonomic and functional diversity decreased. Therefore, our findings illustrate that the invasive *L. fortunei* decreases multiple biodiversity facets of phytoplankton community and negatively affects the functioning (i.e., primary production) of invaded ecosystems.

The observed negative relationship between *L. fortunei* and phytoplankton taxonomic and functional diversity is likely a result of the high filtration rate of the *L. fortunei*. The larvae, although small, can be quite numerous, and their filtration activity can be high, considering that may it is compatible with the filtration activity of adults, which them filter large quantities of suspended particles, including phytoplankton (e.g. Boltovskoy & Correa, 2015). Adults of *L. fortunei* has a filtration rate of approximately 29.5 ml mg h^{-1} at 25 ºC, which is highest compared to another invasive mussels, such as *D. polymorpha* (4.12 ml mg h^{-1} at 22 ºC) and *C. fluminea* (20.5 ml mg h^{-1} at 21 - 24 ºC) (Sylvester et al., 2005). Consequently, *L. fortunei* may drastically decrease phytoplankton diversity and abundance as they accumulate large density. Moreover, *L. fortunei* may select specific traits of the phytoplankton through mechanical filters, such as the size of the inhaling siphon (Vanderploeg et al., 2001), or by selection branchial level (Fachini et al., 2012). Such trait selection appears to have occurred in our study because *L. fortunei* had a positive relationship with those phytoplankton traits related to avoiding and escaping predation, such as the presence of mucilage and siliceous walls and swimming motility (Ger et al., 2016; Graco-Roza et al., 2021). Conversely, the abundance of phytoplankton species without scape ability (e.g., absence of flagellum or floating motility) or characteristics to hinder filtration (e.g., presence of mucilage and siliceous walls, and ability to form colonies and chains) decreased with increasing *L. fortunei* density. Therefore, there is a clear evidence that *L. fortunei* selected phytoplankton traits combinations. This trait selection likely contributes to functional homogenization of the phytoplankton, since they remain in the system only those species with traits that somehow hinder the predation of *L. fortunei*.

As expected, the characteristics related to small dimensions and the inability to escape were most negatively affected by the high larval densities. These characteristics are more easily predated, as they have no mechanical limitations to their intake (Vanderploeg et al., 2001; Fachini et al., 2012), and those without motility has lower probability to escape the flow of water being filtered. In addition, the constant selection of functional traits through the filtration of *L. fortunei*, associated with the high densities of these larvae, can culminate in the excessive loss of species/traits, leading to homogenization of the phytoplankton community. The loss of species and functionality may result in the loss of ecosystem functions (Hooper et al., 2005).

Adults of *L. fortunei* are ecosystem engineers, thus, it may change environment characteristics, which may indirectly affect phytoplankton community (Cataldo et al., 2012a; De Stefano et al., 2018). The dams present in some of the evaluated environments (Baía and Paraná rivers) to negatively affect the availability of nutrients (Roberto et al., 2009), what may be intensified in the presence of *L. fortunei*. We found that *L. fortunei* was related to the decreased in

N:P ratio, which had negative effects on taxonomic richness of the phytoplankton. This effect on the nutrient cycle has already been evidenced for the group of bivalves. In general, these changes are related to direct excretion rates and microbial-mediated remineralization of the produced organic deposits (McKindsey et al., 2006). Cataldo et al. (2012a) observed, experimentally, that the metabolic activity of *L. fortunei* decreases the availability of particulate N and P, while increasing the concentration of NH_3 and PO_4. The decrease in N:P rates shown in our results is probably related to the increase in phosphorus in the water column, due to the metabolic activities of *L. fortunei*. The increase in the concentration of phosphorus can favor the growth of phytoplankton species, especially those that are not limited by the availability of nitrogen, as is the case with cyanobacteria (Smith, 1983; Reynolds, 1987; Steinberg & Hartmann, 1988), which are capable of fixing atmospheric nitrogen (Reynolds, 2006).

The larvae of *L. fortunei* had direct negative effects on the taxonomic and functional richness of phytoplankton, which reflected indirectly on the biomass of the community. Because phytoplankton is one of the main primary producers of freshwater environments, especially in the pelagic zones (Field et al., 1998; Olsen, 2002; Sommer et al., 2002), its biomass can be considered a proxy for primary productivity. The decrease in primary producer's biomass can lead to the decline in the diversity (Field et al., 2008) and in consumers productivity (e.g., Kindeys, 2002). These effects tend to extend to higher trophic levels via trophic cascade (phytoplankton-zooplankton-fish, e.g., Thompson, 2005) and cause not only environmental damage, but also economic and social damage due to diminished fishing resources (e.g., Kindeys, 2002).

The densities of *L. fortunei* larvae vary widely throughout the year and in space (e.g., Pestana et al., 2008; Ernandes-Silva et al., 2017), and the dispersion capacity of phytoplankton species, especially individuals smaller, is quite high (e.g., Fuhrman, 2009; Wilkinson et al. 2011). Therefore, environments receive algae organisms at all times, which can restore the wealth of the community at certain times and places. However, if population control measures are not taken, and the density of *L. fortunei* increases further, especially in more closed and small environments, such as lakes (Amo et al., 2021) the effects on the phytoplankton community can be intensified and the impacts generated on the community become more evident.

We conclude that *L. fortunei* larvae are associated with negative effects on taxonomic and functional diversity of phytoplankton, as well as on community biomass. These effects can extend to higher trophic levels and culminate with ecosystems effects, due to the effects on the diversity, functionality and biomass of the phytoplankton community. Considering that *L. fortunei* has the potential to establish itself in several other regions of the globe (Petsch et al., 2020), and that this species can cause impacts on diversity and the functioning of invaded ecosystems, we suggest that

control measures should be prioritized in order to decrease the density of *L. fortunei*. We hope that our results represent a warning that not only the density, but also the expansion of this invader should be included in control plans. Finally, we emphasize the need for experimental studies in order to strengthen and refine the results obtained here.

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APPENDIX B - Justification of the selection of functional traits, and list of phytoplankton species used to carry out the analyses.

Table 1. Functional traits of phytoplankton that can be selected by the filtration activity of *L. fortunei* larvae, and their implications for the study.

Traits	***Category***	***Implications for the study***
Volume	Continuous	Functional traits related to the size of the cells or cell group can influence the filtering activity of bivalves, given that the dimensions must be compatible with the mechanical filters of the species (size of the inhaling siphon, Ward & Shumway, 2004) and that studies have already shown the preference by small particles (eg Tem Winkel & Davids, 1982; Ward & Shumway, 2004).
Tendency to form chains and colonies	Chains	
	Colonies	
	Non-form	
Mucilage	Dummy	
Flagellate	Dummy	The siliceous wall, found in some phytoplankton species, hinders the digestion of organic matter and access to nutrients, and is commonly released in the form of pseudo-feces (Ward & Shumway 2004).
Motility	Swimming	Motility is a functional trait that relates to the ability to prevent predation, as it allows the mobile organism the ability to move away and / or escape from the predator and / or water stream created by the filtering organisms (Jakobsen, 2001; Harvey & Menden-Deuer, 2012; Pancic & Kiorboe, 2018).
	Gliding	
	Floating	
Siliceous Wall	Dummy	

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Table 2. Taxonomic groups of phytoplankton and their respective categorization in the functional traits evaluated. Tend. Chains or Colon = Tendency to form chains or colonies.

Taxonomic Groups	Volume (μm^3)	Tend. Chains or colon.	Mucilage	Flagellated	Cell motility	Siliceous walls
Acutodesmus acuminatus	123.28	Colony	0	0	floating	0
Ankistrodesmus fusiformes	299.5	Colony	0	0	floating	0
Ankistrodesmus gracilis	1318.25	Colony	0	0	floating	0
Ankyra judayi Fott	190.09	NF	0	0	floating	0
Ankyra sp.	190.09	NF	0	0	floating	0
Chlorococcales unidentified unicellular	113	Colony	1	0	floating	0
Coelastrum proboscideum	1041.3	Colony	0	0	floating	0
Desmodesmus armatus var. *armatus*	277.82	Colony	0	0	floating	0
Desmodesmus armatus var. *bicaudatus*	223.8	Colony	0	0	floating	0
Desmodesmus brasiliensis	277.82	Colony	0	0	floating	0
Desmodesmus communis	1047	Colony	0	0	floating	0
Desmodesmus denticulatus var. *denticulatus*	91.8	Colony	0	0	floating	0
Desmodesmus intermedius var. *acutispinus*	366.52	Colony	0	0	floating	0
Desmodesmus intermedius var. *intermedius*	366.52	Colony	0	0	floating	0
Desmodesmus opoliensis	62.83	Colony	0	0	floating	0
Eutetramorus fottii.	629.2	Colony	1	0	floating	0
Monoraphidium arcuatum	58.8	NF	0	0	floating	0
Monoraphidium circinale	26.4	NF	0	0	floating	0
Monoraphidium contortum	16.5	NF	0	0	floating	0
Monoraphidium convolutum	60.77	NF	0	0	floating	0
Monoraphidium griffithii	114.4	NF	0	0	floating	0

cont.

cont.

Taxonomic Groups	Volume (µm³)	Tend. Chains or colon.	Mucilage	Flagellated	Cell motility	Siliceous walls
Monoraphidium irregulare	44.41	NF	0	0	floating	0
Monoraphidium komarkovae	43.38	NF	0	0	floating	0
Monoraphidium minutum	37.92	NF	0	0	floating	0
Monoraphidium tortile	16	NF	0	0	floating	0
Pediastrum duplex Mey. var. *duplex*	9370	Colony	0	0	floating	0
Quadrigula closterioides	86.82	Colony	0	0	floating	0
Scenedesmus ecornis var. *ecornis*	116.16	Colony	0	0	floating	0
Scenedesmus obtusus	653.89	Colony	0	0	floating	0
Scenedesmus ovalternus	66.72	Colony	0	0	floating	0
Scenedesmus sp.	79.3	Colony	0	0	floating	0
Schroederia antillarum	35.29	NF	0	0	floating	0
Schroederia setigera	94.84	NF	0	0	floating	0
Stauridium tetras	292.8	Colony	0	0	floating	0
Tetraedron minimum	44.18	Colony	0	0	floating	0
(Crucigenia quadrata)	717	Colony	0	0	floating	0
Actinastrum hantzschii	484	Colony	0	0	floating	0
Closteriopsis sp. *(scolia)*	245.24	NF	0	0	floating	0
Crucigenia fenestrata	800.64	Colony	0	0	floating	0
Desmatractum indutum	46.28	Colony	0	0	floating	0
Dictyosphaerium elegans	125.52	Colony	1	0	floating	0
Dictyosphaerium pulchellum	1089	Colony	1	0	floating	0
Lagerheimia ciliata	138.84	Colony	1	0	floating	0
Lemmermannia tetrapedia	179.4	Colony	0	0	floating	0
Micractinium belenophorus	34	Colony	0	0	floating	0
Micractinium pusillum	1600.43	Colony	0	0	floating	0

cont.

cont.

Taxonomic Groups	Volume (µm³)	Tend. Chains or colon.	Mucilage	Flagellated	Cell motility	Siliceous walls
Nephroclamys sp.	356.19	Colony	1	0	floating	0
Oocystis borgei	13825.53	Colony	1	0	floating	0
Brachiogonium ophiaster	156.09	NF	0	0	floating	0
Goniochloris mutica	46	NF	0	0	floating	0
Goniochloris spinosa	312	NF	0	0	floating	0
Tetraplektron acutum	789.81	NF	0	0	floating	0
Tetraplektron tribulus	312.37	NF	0	0	floating	0
Closterium gracile.	255.35	NF	0	0	floating	0
Closterium incurvum	122.1	NF	0	0	floating	0
Closterium setaceum	7636.3	NF	0	0	floating	0
Closterium sp.	2245.48	NF	0	0	floating	0
Cosmarium contractum	2941.67	NF	0	0	floating	0
Cosmarium margaritatum	1570	NF	0	0	floating	0
Cosmarium sp.	1359.53	NF	0	0	floating	0
Micrasterias foliacea	69750	Chains	0	0	floating	0
Mougeotia sp.	2042.83	Chains	0	0	floating	0
Onychonema laeve	123151	Chains	0	0	floating	0
Spyrogira sp.	125718.7	Chains	0	0	floating	0
Staurastrum leptocladum var. *leptocladum*	7524.89	NF	0	0	floating	0
Staurodesmus clepsydra	288	NF	0	0	floating	0
Staurodesmus dejectus	2348.9	NF	0	0	floating	0
Staurodesmus triangularis	9414.14	NF	0	0	floating	0
Teilingia granulata	11444	Chains	0	0	floating	0
Oedogonium sp.	14585.77	Chains	0	0	floating	0
Aphanizomenon gracile	3307	Chains	0	0	floating	0
Aphanocapsa delicatissima	302	Colony	1	0	floating	0
Aphanocapsa holsatica	471.35	Colony	1	0	floating	0
Aphanocapsa sp.	392.7	Colony	1	0	floating	0

cont.

cont.

Taxonomic Groups	Volume (µm³)	Tend. Chains or colon.	Mucilage	Flagellated	Cell motility	Siliceous walls
Aphanothece clathrata	47	Colony	1	0	floating	0
Aphanothece smithii	47.12	Colony	1	0	floating	0
Microcystis	50	Colony	1	0	floating	0
Chroococcus microscopicus	31.42	Colony	1	0	floating	0
Chroococcus minutus	28.27	Colony	1	0	floating	0
Coelomoron tropicale	69.46	Colony	1	0	floating	0
Cyanodictyon imperfectum	301.59	Colony	1	0	floating	0
Cyanodictyon reticulatum	301	Colony	1	0	floating	0
Cyanodictyon sp.	73.9	Colony	1	0	floating	0
Cyanogranis ferruginea	9.2	Colony	1	0	floating	0
Cylindrospermopsis sp.	2537.5	Chains	1	0	floating	0
Dolichospermum circinalis	8527.4	Chains	0	0	gliding	0
Dolichospermum planctonicum Anabaena planctonica	31417.84	Chains	0	0	gliding	0
Dolichospermum spiroides	7150	Chains	0	0	gliding	0
Geitlerinema sp.	573.03	Chains	0	0	floating	0
Konvophoron sp.	80.1	Chains	0	0	floating	0
Lemmermanniella pallida	431	Colony	1	0	floating	0
Lyngbya sp.	1204.6	Chains	0	0	floating	0
Merismopedia tenuissima	34	Colony	0	0	floating	0
Microcystis aeruginosa	27043.8	Colony	0	0	gliding	0
Microcystis novacekii	20052.5	Colony	0	0	gliding	0
Microcystis panniformis	610.7	Colony	0	0	gliding	0
Microcystis sp.	1650.88	Colony	0	0	gliding	0
Oscillatoria sp.	26938.18	Chains	0	0	floating	0
Pannus sp.	471.35	Colony	1	0	floating	0
Planktothrix agardhii	2120	Chains	0	0	floating	0

cont.

cont.

Taxonomic Groups	Volume (µm³)	Tend. Chains or colon.	Mucilage	Flagellated	Cell motility	Siliceous walls
Pseudanabaena limnetica	42.5	Chains	0	0	floating	0
Pseudanabaena mucicola	36.01	Chains	0	0	floating	0
Pseudanabaena sp.	25.6	Chains	0	0	floating	0
Pseudanabaenaceae unidentified 1	480.42	Chains	0	0	floating	0
Pseudanabaenaceae unidentified 3	35	Chains	0	0	floating	0
Radiocystis fernandoi	70324.26	Colony	0	0	gliding	0
Romeria gracilis	26.05	Chains	0	0	floating	0
Romeria sp.	26.05	Chains	0	0	floating	0
Snowella atomus	54	Colony	1	0	floating	0
Achnantes exigua	79.9	Chains	0	0	floating	1
Achnantes sp.	78.39	Chains	0	0	floating	1
Achnanthidium minutissimum	79.9	Chains	0	0	floating	1
Amphipleura lindheimeri.	14157.53	Chains	0	0	swimming	1
Amphora sp.	617.9	Chains	0	0	swimming	1
Cocconeis sp.	516.5	Chains	0	0	swimming	1
Cymbella sp.	201.96	Chains	0	0	swimming	1
Encyonema silesiacum	642.5	Chains	0	0	swimming	1
Eunotia cf. *tukanorum*	78.39	Chains	0	0	swimming	1
Eunotia didyma Grun. var. *curta*	324.29	Chains	0	0	swimming	1
Eunotia didyma Grun. var. *didyma*	912	Chains	0	0	swimming	1
Eunotia flexuosa.	1577.277	Chains	0	0	swimming	1
Eunotia longicamelus	1463	Chains	0	0	swimming	1
Eunotia minor	300	Chains	0	0	swimming	1
Eunotia paludosa	480	Chains	0	0	swimming	1
Eunotia sp.	324.9	Chains	0	0	swimming	1
Fragilaria longifusiforme	125	Chains	0	0	swimming	1
Fragilaria longifusiformes	528.71	Chains	0	0	swimming	1
Fragilaria sp.	6837.84	Chains	0	0	swimming	1
Gomphonema augur	730	Chains	0	0	swimming	1

cont.

cont.

Taxonomic Groups	Volume (µm³)	Tend. Chains or colon.	Mucilage	Flagellated	Cell motility	Siliceous walls
Gomphonema brasiliense	580.11	Chains	0	0	swimming	1
Gomphonema gracile	1141.5	Chains	0	0	swimming	1
*Gomphonema olivaceum*t	580.11	Chains	0	0	swimming	1
Gomphonema parvulum	189.75	Chains	0	0	swimming	1
Gomphonema sp.	580.11	Chains	0	0	swimming	1
Navicula sp.	305.5	Chains	0	0	swimming	1
Nitzschia palea	234.5	Chains	0	0	swimming	1
Nitzschia sp.	403.6	Chains	0	0	swimming	1
Pennales unidentified 1	1438.88	Chains	0	0	swimming	1
Pennales unidentified 8	331.5	Chains	0	0	swimming	1
Pinnularia sp.	1912.5	Chains	0	0	swimming	1
Stauroneis sp.	1204.6	Chains	0	0	swimming	1
Surirella apiculata	3094	Chains	0	0	swimming	1
Surirella guatimalensis	6188	Chains	0	0	swimming	1
Surirella sp.	6188	Chains	0	0	swimming	1
Surirella sp1	125	Chains	0	0	swimming	1
Surirella tenera Greg. var. *nervosa*	6188	Chains	0	0	swimming	1
Synedra goulardi	10069.23	Chains	0	0	swimming	1
Synedra sp.	1501.11	Chains	0	0	swimming	1
Ulnaria ulna.	8851.03	Chains	0	0	floating	1
Aulacoseira ambigua var. *ambigua spiralis*.	6503.11	Chains	0	0	floating	1
Aulacoseira distans	620.87	Chains	0	0	floating	1
Aulacoseira granulata var. *angustissima*	567.59	Chains	0	0	floating	1
Aulacoseira granulata var. *angustissima curvata*	567.59	Chains	0	0	floating	1
Aulacoseira granulata var. *granulata*	7454.81	Chains	0	0	floating	1
Aulacoseira herzogii	4707.69	Chains	0	0	floating	1

cont.

cont.

Taxonomic Groups	Volume (µm³)	Tend. Chains or colon.	Mucilage	Flagellated	Cell motility	Siliceous walls
Aulacoseira muzzanensis Krammer	7500	Chains	0	0	floating	1
Aulacoseira sp.	883.58	Chains	0	0	floating	1
Aulacoseira sp1	94.25	Chains	0	0	floating	1
Cyclotella meneghiniana	168.9	Chains	0	0	floating	1
Cyclotella sp.	144.2	Chains	0	0	floating	1
Discostella stelligera	120.17	Chains	0	0	floating	1
Urosolenia eriensis eriensis	1564.27	Chains	0	0	floating	1
Urosolenia eriensis var. *morsa*	1564.27	Chains	0	0	floating	1
Urosolenia longiseta	925.8	Chains	0	0	floating	1
Urosolenia sp.	925.8	Chains	0	0	floating	1
Acanthoceras magdeburgensis Hongimann	6048.6	Chains	0	0	floating	1
Chlamydomonas sp.	95.44	Chains	0	1	swimming	0
Chlamydomonas sp1	628.32	Chains	0	1	swimming	0
Chlamydomonas sp2	150.8	Chains	0	1	swimming	0
Chlamydomonas sp3	255	Chains	0	1	swimming	0
Chlorogonium cf. *fusiforme*	27.5	Chains	0	1	swimming	0
Eudorina sp.	5424.6	Chains	0	1	swimming	0
Flagelado	91.63	Chains	0	1	swimming	0
Gonium cf. *pectorale* Müll.	3516.21	Chains	0	1	swimming	0
Lobomonas sp.	95.44	Chains	0	1	swimming	0
Pandorina morum	555.36	Chains	0	1	swimming	0
Pteromonas variabilis	157.08	Chains	0	1	swimming	0
Spermatozopsis exsultans	11.22	Chains	0	1	swimming	0
Sphaerellopsis sp.	131.9	Chains	0	1	swimming	0
Synura	282.7	Chains	0	1	swimming	0
Dinobryon bavaricum	12566.4	Chains	0	1	swimming	0
Dinobryon divergens	11838.22	Chains	0	1	swimming	0

cont.

cont.

Taxonomic Groups	Volume (µm³)	Tend. Chains or colon.	Mucilage	Flagellated	Cell motility	Siliceous walls
Dinobryon sertularia	11838.22	Chains	0	1	swimming	0
Kephyrion littorale	87	Chains	0	1	swimming	0
Kephyrion sp.	102.6	Chains	0	1	swimming	0
Mallomonas cf. *akrokomos*	53.25	Chains	0	1	swimming	0
Mallomonas sp.	282.75	Chains	0	1	swimming	0
Mallomonas sp1	226.2	Chains	0	1	swimming	0
Synura sp.	1340.32	Chains	0	1	swimming	0
Chroomonas sp. (acuta)	41.8	Chains	0	1	swimming	0
Cryptomonas brasiliensis	44.53	Chains	0	1	swimming	0
Cryptomonas curvata	230.91	Chains	0	1	swimming	0
Cryptomonas erosa	230	Chains	0	1	swimming	0
Cryptomonas marssonii	44.53	Chains	0	1	swimming	0
Cryptomonas sp.	42	Chains	0	1	swimming	0
Ceratium	14660.6	Chains	0	1	swimming	0
Peridinium sp.	36954.66	Chains	0	1	swimming	0
Peridinium sp1	3556.15	Chains	0	1	swimming	0
Peridinium sp2	651.15	Chains	0	1	swimming	0
Peridinium sp3	3556.15	Chains	0	1	swimming	0
Peridinium sp4	1642	Chains	0	1	swimming	0
Peridinium umbonatum	1256.22	Chains	0	1	swimming	0
Euglena acus var. *acus*	883.58	Chains	0	1	swimming	0
Euglena sp.	837.76	Chains	0	1	swimming	0
Euglena sp3	163.03	Chains	0	1	swimming	0
Euglena sp4	27.61	Chains	0	1	swimming	0
Euglenophyceae unidentified	196.35	Chains	0	1	swimming	0
Monomorphina pyrum	353.43	Chains	0	1	swimming	0
Monomorphina sp.	353.43	Chains	0	1	swimming	0
Phacus horridus	617.38	Chains	0	1	swimming	0
Strombomonas scabra	600.83	Chains	0	1	swimming	0
Strombomonas subcurvata	600	Chains	0	1	swimming	0
Strombomonas verrucosa	506	Chains	0	1	swimming	0

cont.

cont.

Taxonomic Groups	Volume (µm³)	Tend. Chains or colon.	Mucilage	Flagellated	Cell motility	Siliceous walls
Trachelomonas armata var. *nana*	54.52	Chains	0	1	swimming	0
Trachelomonas dastuguei Balech.	628.53	Chains	0	1	swimming	0
Trachelomonas rugulosa	2006.04	Chains	0	1	swimming	0
Trachelomonas similis var. *similis*	518.36	Chains	0	1	swimming	0
Trachelomonas similis var. *spinosa*	518.36	Chains	0	1	swimming	0
Trachelomonas sp1	1847.26	Chains	0	1	swimming	0
Trachelomonas volvocinopsis Swir.	1085	Chains	0	1	swimming	0
Gonyostomum sp.	2000	Chains	0	1	swimming	0
Gonyostomum sp1	184	Chains	0	1	swimming	0
Gonyostomum sp2	942.48	Chains	0	1	swimming	0
Raphidoficea unidentified	7539.9	Chains	0	1	swimming	0

4 FINAL CONSIDERATIONS

Invasive species cause a variety of impacts around the globe. Many of the impacts related to these species are due to their high densities, and consequent dominance in the invaded communities. Thus, knowing the factors that affect the establishment and population growth of these species can assist in environmental management such as in the identification of areas most vulnerable to invasion. Here, we evaluated the environmental filters that act on each of the larval stages of *L. fortunei*, and contribute to the age structure of the species population in different lotic environments. In addition, we evaluated the potential effects of *L. fortunei* larval density on the diversity facets and phytoplankton, and consequently, on the community's biomas stocks.

We identified that the age pyramid of *L. fortunei* in the upper Paraná River floodplain indicates that the population of *L. fortunei* in the region is still growing, and that the environmental filters that most affect the larval stages are concentrated in the initial stages. A difference was observed in the age pyramids of the different environments evaluated, which indicates that certain more degraded rivers are more propitious to reproduction (Baia and Paraná rivers), while others (Ivinhema River) favor the development and survival of the larvae. Among the identified filters, we highlight the positive effect of temperature, which tends to favor an increase in the density of larvae in a scenario of global warming. We also identified the negative effect of turbidity, which tends to be reduced as a result of the installation of multiple reservoirs, and consequently, may further favor the survival of the larval stages of *L. fortunei*

The high larval density of *L. fortunei* was associated of negative effects on the taxonomic and functional diversity of the phytoplankton community, which culminated in indirect effects on the biomass stock of the community. Considering that the phytoplankton community is the main primary producer of aquatic environments, and that it serves as food for the base of aquatic trophic chains, it is expected that this effect will reach ecosystem levels.

We hope that our results will alert about the possible impacts that *L. fortunei* can cause on the phytoplankton community, and indirectly, on the ecosystem functioning. We emphasize that these impacts may become more evident if the population control of this species does not occur and its density increases even more especially in scenarios of global warming and dammed rivers.