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Limnoperna fortunei Dunker, 1857 larvae in different environments of a Neotropical floodplain: relationships of abiotic variables and phytoplankton with different stages of development

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(With 6 figures)

Abstract

Limnoperna fortunei Dunker, 1857 is an Asian invasive freshwater bivalve. Although there need to contain their spread, studies about the biology of the larvae are scarce. We correlated the larval stages of *L. fortunei* with biotic factors such as phytoplankton and main abiotic variables in lotic environments of the Upper Paraná River floodplain. The four samples were taken quarterly during the year 2012. The Principal component analysis (PCA) showed only spatial differences, as did a Canonical Correspondence Analysis (CCA). High densities of larvae were recorded in all samples the Paraná River and Baía River only in December, especially those in their initial stage. In the biovolume of Class of algae, Bacillarophyceae showed the highest value, but Chlorophycea who was strongly correlated with the density of D-stage larvae. The large variety of phytoplankton, especially microplankton Chlorophyceae, high values of PO_4, NH_4 and temperature were positively correlated with high densities of D-stage larvae. We conclude that high temperature, and food availability, indicated by phytoplankton community, favored the reproduction of *L. fortunei* and enhance the ability of specie dispersion due to the increase in the emission of propagules. Therefore, studies that address the biology of golden mussel larvae should be performed in order to prevent its spread.

Keywords: invasive species, limnic bivalves, Paraná river, dispersal patterns, environmental filters.

Larvas de *Limnoperna fortunei* Dunker, 1857 em diferentes ambientes de uma planície de inundação Neotropical: relação com variáveis abióticas e algas fitoplanctônicas em diferentes estágios de desenvolvimento

Resumo

Limnoperna fortunei Dunker, 1857 é um bivalve asiático dulcícola invasor. Embora haja necessidade de conter sua dispersão, estudos que abordam a biologia de suas larvas ainda são escassos. Analisou-se as fases larvais de *L. fortunei* relacionando-as a fatores bióticos como a comunidade fitoplânctônica e às principais variáveis abióticas, em ambientes lóticos da planície de inundação do alto do rio Paraná. As quatro coletas foram trimestrais durante o ano de 2012. A análise de componentes principais (PCA) demonstrou apenas diferenças espaciais, assim como a Análise de Correspondência Canônica (CCA). Altas densidades de larvas foram registradas em todas as coletas no rio Paraná e no rio Baía apenas no mês de dezembro, principalmente para as larvas em estádio inicial. No biovolume das classes de algas, Bacillarophyceae obteve o maior valor, porém Chlorophycea foi a que fortemente correlacionou-se com a densidade de larvas D. A grande variedade de fitoplâncton, em especial de Chlorophyceae microplanctônica, altos valores de PO_4, NH_4 e temperatura estiveram positivamente correlacionadas com altas densidades de larvas D. Conclui-se que, altas temperaturas, e disponibilidade de alimento, como a comunidade fitoplanctônica, favorecem a reprodução de *L. fortunei* e aumentam a capacidade de dispersão da espécie devido ao incremento na emissão de propágulos. Portanto, estudos que abordem a biologia das larvas de mexilhão-dourado devem ser realizados a fim de evitar sua propagação.

Palavras-chave: espécie invasora, bivalves límnicos, rio Paraná, padrão de dispersão, filtros ambientais.

1. Introduction

Floodplains like the Upper Paraná River, are characterized by high heterogeneity of habitats (Ward et al., 2002; Ribeiro et al., 2012) and consequently high species diversity. Inspite of high levels of biodiversity, reservoir cascades located upstream of Upper Paraná River floodplain (Souza Filho and Stevaux, 1997) have caused several changes in the hydrological regime and the physical and chemical water parameters (Agostinho et al., 2013). Such changes have strongly affected the aquatic communities (Barletta et al., 2010).

Concomitant to these changes, many invasive species have arrived emerged such as: *Urochloa subquadripara* (Trin.) R.D. Webster; *Hydrilla verticillata* (L.F.) Royle; *Daphnia lumholtzii* Sars, 1885; *Corbicula fluminea* Müller, 1774 and *Limnoperna fortunei* Dunker, 1857. Among these, *Limnoperna fortunei* commonly presents a high abundance and wide distribution in lotic environments of this floodplain (Agostinho et al., 2013).

Limnoperna fortunei Dunker, 1857, popularly known as golden mussel, is a freshwater bivalve (Darrigran and Ezcurra de Drago, 2000) introduced into South America by vessel ballast water (Mansur et al., 1999; Darrigran and Pastorino, 1995). In Brazil, the first record was in 1999 (Mansur et al., 1999) in Rio Grande do Sul (Mansur et al., 2003), with subsequent registers in aquatic environments of different states as São Paulo (Avelar et al., 2004), Mato Grosso do Sul and Mato Grosso (Darrigran and Pastorino, 2003; Oliveira et al., 2006) and Paraná (Takeda et al., 2003).

The invasion success of this species is intrinsically related to the biological characteristics of its life cycle (Lodge et al., 1998). A planktonic larval stage and an adult phase with encrusting capacity ensure a great spreading ability and potential of colonizing new areas. Initial stages of development are easily carried by water flow to new environments, while the latter stages tend to disperse due to traffic of vessels between connected water bodies. Among these phases, Oliveira et al. (2004) consider that the larval stage is the most favorable for dispersion.

This specie feeds on suspended material in the water column (Sylvester et al., 2009), such as the zooplankton community and, especially, the phytoplankton community (Fachini et al., 2012). Although the latter represents a small fraction of the diet of *L. fortunei*, phytoplankton is responsible for supplying more than 90% of the energy demand required by the specie at certain seasons (Sylvester et al., 2009). In addition to food resources, some abiotic factors seem to facilitate the establishment of *L. fortunei*, such as oxygen content and water temperature (Darrigran and Pastorino, 1995; Boltovskoy and Cataldo, 1999; Mansur et al., 2003; Cataldo et al., 2005; Darrigran et al., 2007; Pestana et al., 2010).

The objective of this study was to investigate the distribution of *L. fortunei* larvae and correlate their abundance with phytoplankton community and abiotic factors. We tested the hypothesis that the availability of palatable phytoplankton and favorable abiotic factors contributes to high densities of *L. fortunei* larvae. Our predictions were: (i) environments with high densities of algae and high values of dissolved oxygen and temperature will present higher densities of *L. fortunei* larvae (ii) environments with higher densities of nanophytoplankton will have higher density of larvae in early stages as D and Straight-hinged larvae.

2. Material and Methods

2.1. Study area

This study was conducted in the Upper Paraná River floodplain (Paraná, Brazil). The sampling area stretched from cities Três Lagoas (Mato Grosso do Sul, Brazil) to Guaíra (Paraná, Brazil), between the Porto Primavera and Itaipu dams, approximately 230 km and cover an area of 526,752 ha (Souza Filho and Stevaux, 1997). Samples were collected at 5 stations (Figure 1).

The stretch of the floodplain presents a braided channel, with high levels of current flow, and channel of varying widths with extensive islands (Pauleto et al., 2009). The mean depth of the river is about 4.0 m and may reach 15.0 m at this stretch (Thomaz et al., 1992). Samples were taken in the Paraná River (22° 75' 68" - 53° 25' 31"), Ivinhema River (22° 51' 23" S - 53º 36' 23" W) and Ipoitã Channel (22° 83' 59" - 53° 56' 37"). This channel which connects the Paraná and Ivinhema rivers is one of main tributaries situated on the right bank of the Paraná River (Pauleto et al., 2009).Samples were also taken in the Baía River (22° 72' 31" - 53° 29' 04") which is connected to the Paraná River through the Baía Channel. In addition to the main channel, samples were taken in Curutuba Channel (22° 75' 32" - 53° 35' 85") which connects Baia River to Ivinhema River (Pauleto et al., 2009). Baía River is a lentic water environment with varied width, a mean depth of 3.2 m, low declivity and low current flow and is directly influenced by the hydrological regime of Paraná River (Thomaz et al., 1992).

2.2. Sampling

Samples of larval density were taken in March, June, September and December of 2012, in the central regions of Paraná, Ivinhema and Baía rivers and Ipoitã and Curutuba channels. At each station we filtered three samples of 100 L in the water column (approximately 0.5m deep), with plankton net with mesh size of 30 µm. After filtration, the material was fixed in 80% alcohol.

Concomitantly, we took measurements at each sampling point of the following variables: water temperature (°C), turbidity (NTU), PO_4- phosphate (µg/L); NO_3-nitrate (µg/L), NH_4- ammonia (µg/L); SOM: suspended organic matter (mg/L); SIM= suspended inorganic matter (mg/L), electric conductivity (µS/cm), D.O= dissolved oxygen (mg/L) provided by the laboratory of Limnology NUPÉLIA.

The water level of the river was taken daily by reading a ruler installed at the margins of Paraná River. Measurements were taken in the morning and afternoon, resulting in a daily average, which were later transformed into monthly averages for some analyzes. The river level

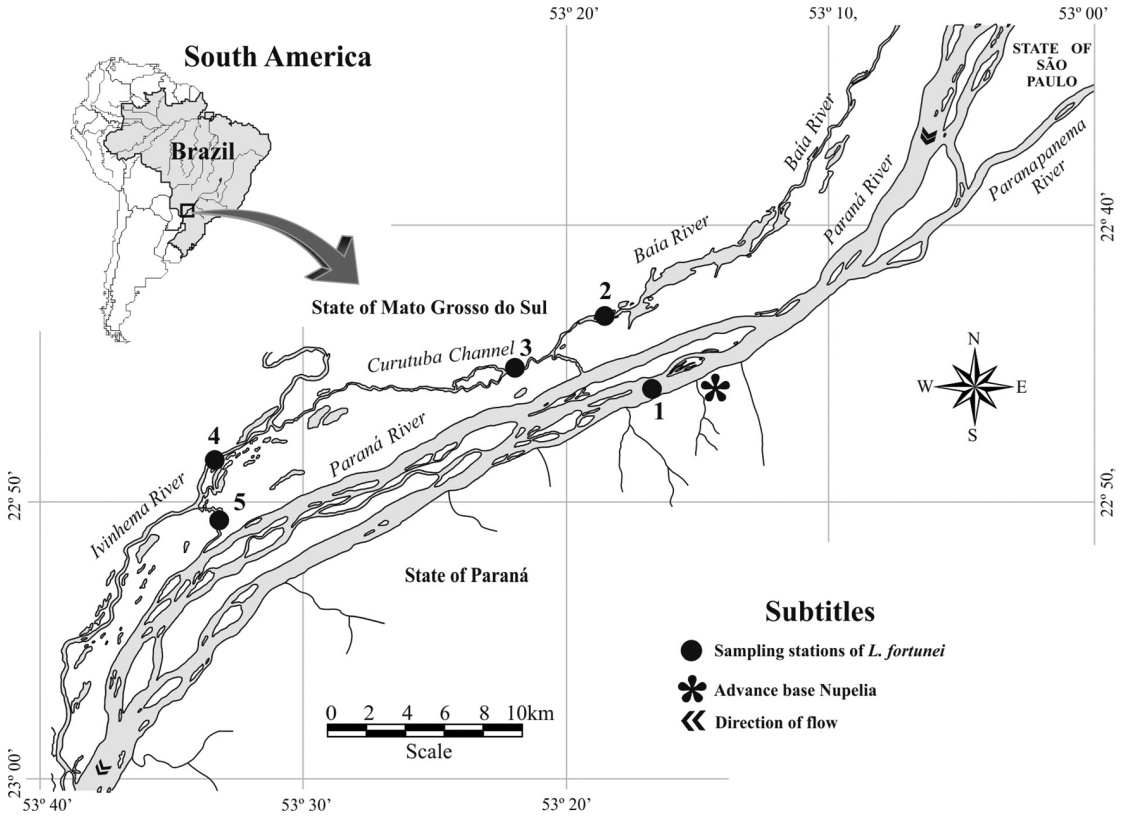

Figure 1. Sampling stations in the Paraná River floodplain. (1) Paraná River (main channel); (2) Baía River (main channel); (3) Curutuba Channel (secondary channel); (4) Ivinhema River (main channel); (5) Ipoitã Channel (secondary channel).

data were expanded for the all environments because the Paraná River constantly influences these areas.

In the laboratory, larvae of *L. fortunei* were counted under stereo microscope. The abundance of organisms was evaluated by full counting, except in samples presenting extremely high densities of individuals, in which we realized three subsequent sub-samplings obtained with Hensen Stempell pipettes (2.5 mL).

Larvae valves of *L. fortunei* were measured for length and width and classified into larvae stages according to the methods adopted by Santos et al. (2005) and Ezcurra de Drago et al. (2006). Furthermore, for the analysis, we classified larvae into five categories according to the size of their valves: D (90-130 µm), Straight-hinged (140-180µm), Umbonated (190-220 µm), Pediveliger (230-270 µm) and Plantigrader (280-490 µm) (Figure 2).

Sampling of phytoplankton was carried out directly at the water's subsurface (20 cm depth). Phytoplankton samples were preserved with acidified Lugol's solution. The phytoplankton density was estimated according to Utermöhl (1958) and Lund et al. (1958), and the species richness was considered as the number of taxa present in one hundred fields of microscope in each quantitative sample. In the case of samples with low density algae, we used the method of curve stabilization. The phytoplankton biovolume was estimated by multiplying the density of different taxa by their respective volumes. The volume of each cell was calculated from geometric models approximating the form of individuals (Sun and Liu, 2003).

2.3. Statistical analysis

To make the graphic of hydrometric levels of the river and the periods of high and low water we used the average of the measurements (morning and afternoon) of the Paraná River, at the base of Porto Rico-PR provided by the NUPÉLIA.

To verify the influence of abiotic variables, we used a principal component analysis (PCA), using PC-ORD 5.0 software (McCune and Mefford, 1999). Axes were retained for interpretations according to the Broken-Stick criteria. The variables used in the ordination were depth (m), pH, temperature (°C), conductivity (µS/cm), and dissolved oxygen (mg/L), turbidity (NTU), Secchi (m), suspended inorganic matter- SIM (mg/L), suspended organic matter – SOM (mg/L), NT = total nitrogen (µg / L), PT = total phosphorus (µg / L), PO_4 (µg/L), NO_3 (µg/L), NH_4 (µg/L), measured at Nupélia.

To summarize the biotic and abiotic data we used the Canonical Correspondence Analysis (CCA) (Ter Braak, 1986; Legendre and Legendre, 1998). This analysis is a multivariate ordination performed to verify the grouping between points and identify the factors that most influence axes.

3. Results

The water level had two high periods in January and June, and the samples were taken after these periods of floods. According to Souza Filho (2009), when the level of the Paraná River is below 3.5 meters, it is considered a period of low water for all environments of the floodplain, therefore our sampling were made in the dry seasons (Figure 3).

The PCA results of explained 65.4% (axis 1 = 41.8% and axis 2 = 23.6%), and showed that no temporal differences between samplings. The results also evidenced a clear distinction of three spatial groups. The first one included Baía River and Curutuba Channel, which was influenced by PT (total phosphorus) and PO_4; the second one included Paraná River and Ipoitã Channel that correlated with dissolved oxygen (D.O); and finally an isolated group

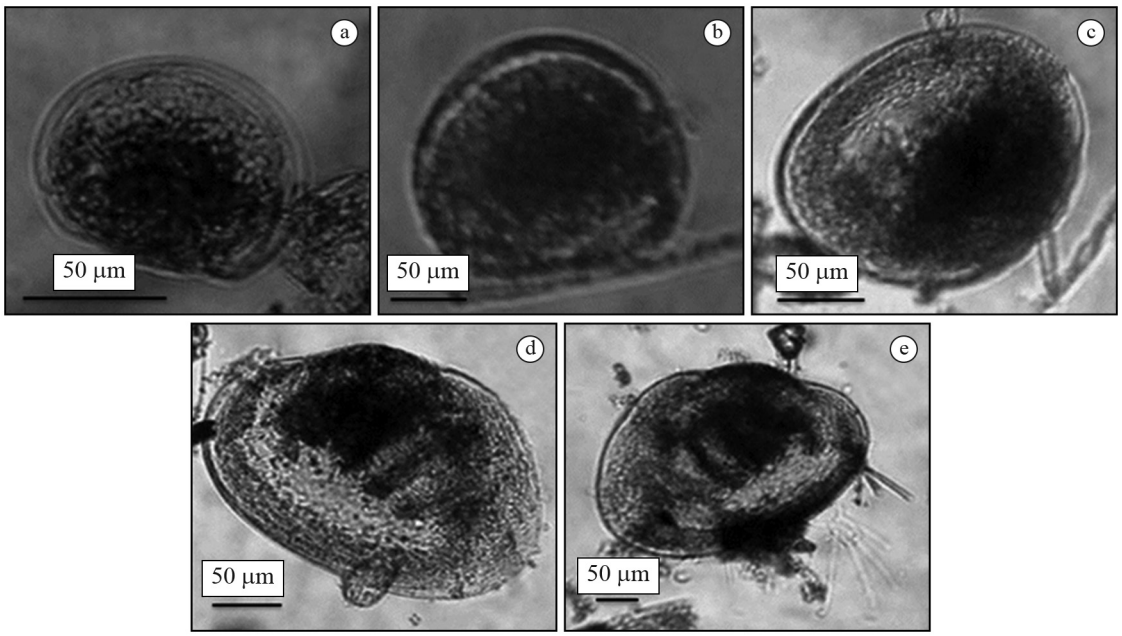

Figure 2. Larval stages with the scale bars. (a) D larvae, 90-130 µm; (b) Straight-hinged, 140-180µm; (c) Umbonated, 190-220 µm; (d) Pediveliger, 230-270 µm; (e) Plantigrader, 280-490 µm.

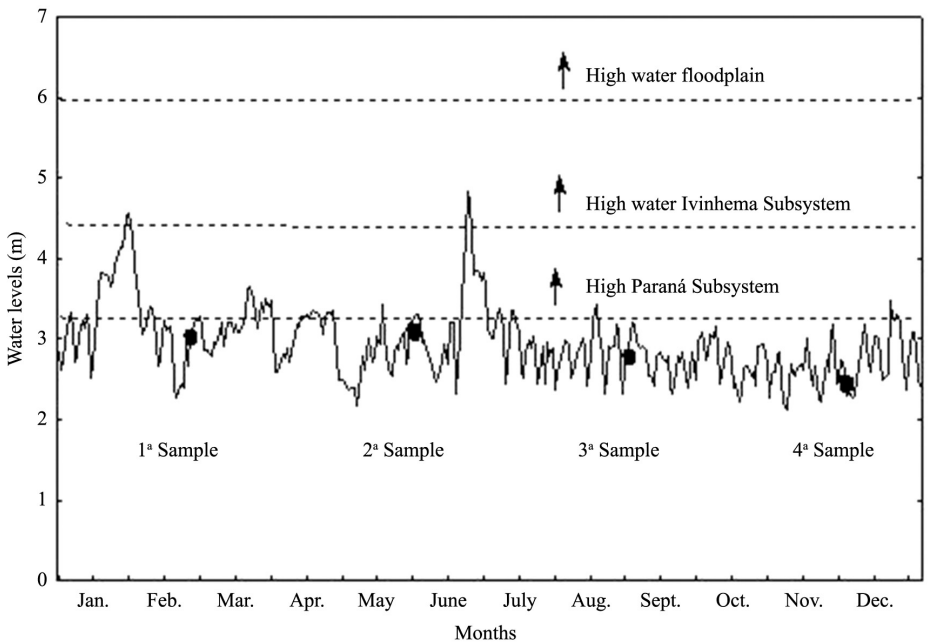

Figure 3. Daily data of the river Paraná level (m) in 2012, obtained from the fluviometric station of Porto Rico-PR, the research base of the NUPÉLIA.

of samples from Ivinhema River which was correlated with turbidity and suspended inorganic materials (SIM) (Figure 4).

The larvae density distribution of *L. fortunei* follows a temporal pattern, in which the highest densities were recorded in December, particularly Baía River (Figure 5a). Algae densities did not show any temporal pattern. The highest densities were recorded in the Paraná river and Baía River in September (BAI-S) and Baía River in December (BAI-D) with the highest density values for the Bacillariophyceae microplankton and Bacillariophyceae nanoplankton (Figure 5b).

Considering the Canonical Correspondence Analysis (CCA), the two first axes were significant ($p < 0.05$) explaining 84% (Figure 6). There was a correlation between the first larval stage (D Larvae) with some abiotic factors, such as temperature, PO_4 and phytoplankton density (Figure 6a). Phytoplankton species were composed primarily by Cholorophyceae and Zygnemaphyceae microphytoplankton (63-500 µm) (Figure 6c) in the environments Baia River in March (BAI-M), Baía River in December (BAI-D), Paraná River in December (PAR-D) and Curutuba Channel in December (CUR-D) (Figure 6b). The other larval stages (Straight-hinged, Umbonated, Pediveliger and Plantigrader) correlated primarily with conductivity, pH, NO_3 and only the Dinophyceae nanoplankton (2-63 µm) (Figures 6a, 6b and 6c).

4. Discussion

Our initial hypothesis was corroborated, because the period of reproductive activity of *L. fortunei* was positively correlated with high densities of phytoplankton, although there is no evidence that this correlation is direct. The presence of dissolved nutrients may have favored the proliferation of phytoplankton and high temperature in December probably induced the reproduction of the golden mussel.

Thus, the phytoplankton community may have contributed indirectly to the high densities of D larvae, due to high food source for adults of the species. Studies about the diet of this bivalve using the plankton community were performed by Pace et al. (1998), Thorp and Casper (2002), Rojas Molina et al. (2010), which evidenced that the availability of food is intimately related with the reproduction of *L. fortunei.*

The principal component analysis (PCA) did not show temporal differences between samples but a spatial difference between environments was verified. The absence of temporal differences in abiotic variables was because the samples were not taken in high water periods.

The Canonical Correspondence Analysis (CCA) showed a clear separation between the first larval stage and the

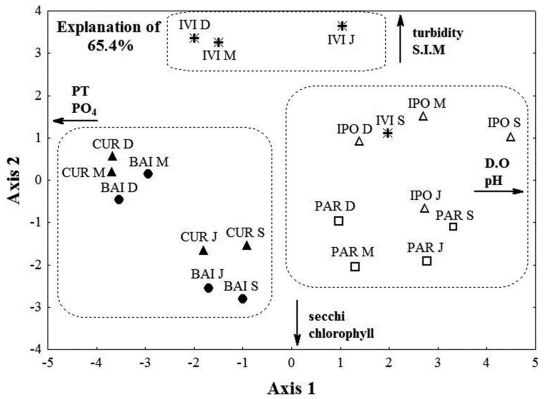

Figure 4. Principal component analysis (PCA) of environments and abiotic variables. BAI= Baía River, IVI= Ivinhema River, PAR= Paraná River, CUR= Curutuba Channel e IPO= Ipoitã Channel (M= March, J= June, S= September e D= December) and (PT= total phosphorus, S.I.M= suspended inorganic material, D.O= dissolved oxygen).

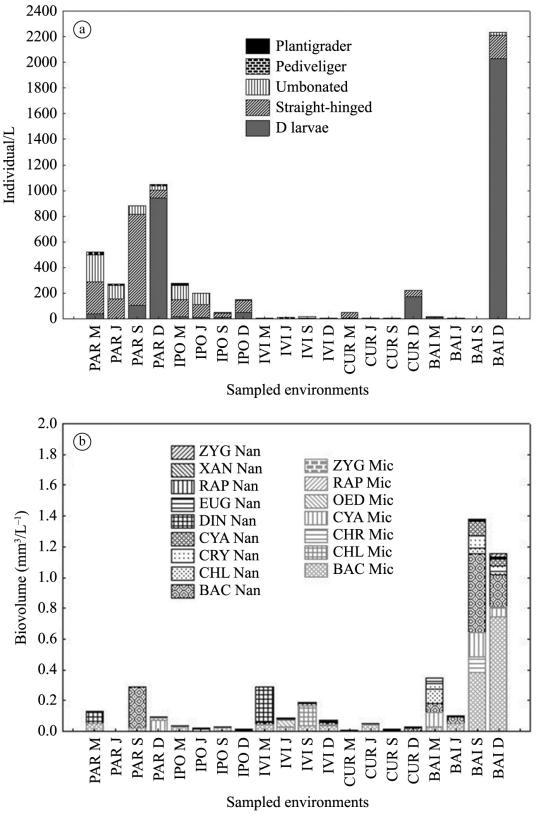

Figure 5. Larvae density (five stages) of *L. fortunei* in the sampled environments of the Upper Paraná River floodplain. (a) Algae density in the sampled environments of Upper Paraná River floodplain; (b) PAR= Paraná River; IPO= Ipoita channel; IVI= Ivinhema River; CUR= Curutuba channel; BAI= Baía River, M= March; J= June; S= September D= December; Mic= microphytoplankton; Nan= nanophytoplankton; BAC= Bacillariophyceae, CYA= Cyanobacteria, CHL= Chlorophyceae, CHY= Chrysophyceae, EUG= Euglenophyceae, CRY= Cryptophyceae, ZYG= Zygnemaphyceae, XAN= Xanthophyceae, DIN= Dinophyceae, RAP= Raphyceae, OED= Oedogoniophyceae.

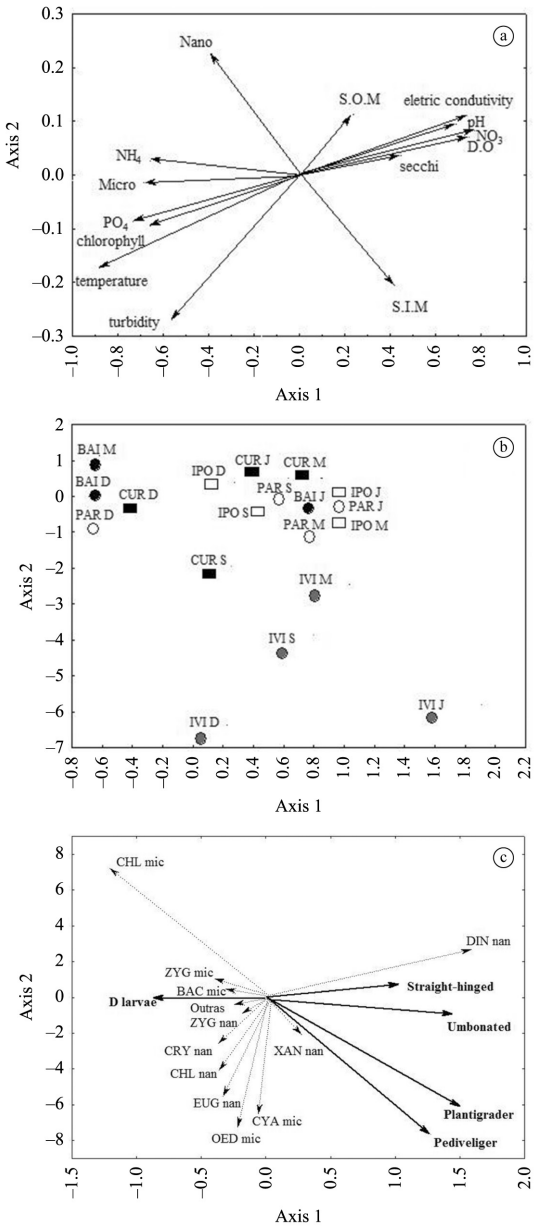

Figure 6. Canonical Correspondence Analysis (CCA). (a) Correlation of abiotic variables: temperature, turbidity, chlorophyll, PO_4 (phosphate); NO_3 (nitrate), NH_4 (ammonia); S.O.M: suspended organic matter; S.I.M=suspended inorganic matter, conductivity, D.O=dissolved oxygen; (b) Correlation of the environments and months (samples). PAR=Paraná River; IPO=Ipoita channel; IVI=Ivinhema River; CUR=Curutuba channel; BAI=Baía River, M=March; J= June; S= September D=December; (c) Correlation of algae and larval stages. BAC=Bacillariophyceae, CYA=Cyanobacteria, CHL=Chlorophyceae, CHY=Chrysophyceae, EUG=Euglenophyceae, CRY=Cryptophyceae, ZYG=Zygnemaphyceae,XAN=Xanthophyceae, DIN=Dinophyceae, RAP= Raphyceae, OED=Oedogoniophyceae, Micro= microphytoplankton; Nano= nanophytoplankton.

others. The first stage was correlated with temperature and the other stages with the high concentration of dissolved oxygen, conductivity and pH. Studies by Cataldo et al. (2005), Darrigran et al. (2007), Kimura and Sekiguchi (1996), Cataldo and Boltovskoy (2000) and Oliveira et al. (2011) suggest that temperature is fundamental for the reproduction, while water oxygenation is important to the development of individuals.

In this way, the high temperatures of the studied region may favor the golden mussel population, due to the constant emission of propagules. In the tropical region average temperatures are higher than in the home range of *L. fortunei*, for example in China where the average maximum is about 27.8 °C (Ho et al., 2003).

Considering the results we obtained, our first prediction was satisfied, that is, the reproduction of *L. fortunei* was correlated with phytoplankton and high temperatures. However, the relationship of the reproduction with the values of dissolved oxygen was not found. This variable was positively correlated with the other larval stages, which shows its importance for larval development, and not for the reproduction of the species.

Among the groups of algae correlated with higher densities of D larvae, the most important was Chlorophyceae, probably because this group of algae is rich in assimilates and does not produce toxins (Happey-Wood, 1988). Thus, the nutritional contribution during this period may be even stronger for the reproduction of *L. fortunei*, since the abiotic factors such as temperature during this period were favorable.

Although high densities of Bacillariophyceae coincided with the period of reproduction, they were not correlated with high larvae densities. The explanation for this may be in these algae silica capsules (Sommer, 1988), which hinder their digestion. This group of algae are difficult to digest, and has been reported in golden mussel pseudofeces (Fachini et al., 2012).

The D Larvae represent the first valved larval stage, and are able to filter their food (Darrigran and Damborenea, 2009). The small sizes of the particles are selected by their siphons, primarily by size (Vanderploeg et al., 2001). Thus, the correlation between the larval stage and nanophytoplankton may favor the development of these larvae. This corroborates the second prediction, but only for D larvae, and the correlation was not found for the straight-hinged stage.

We found a strong correlation of D larvae with nanoplanktonic and microplanktonic algae. This can be explained by the influence of both sizes of algae on the adults of the species. In other words, both the micro and the nanophytoplankton contribute to high densities of D larvae by favoring high reproductive rates of adults.

The results showed high values for the larval density, but the most significant numbers are restricted to early stages. Therefore, the mortality rate seems high, since the large number of D larvae and straight-hinged indicates that there are places where the population is established and continually reproducing. This is alarming, because the

stability of an invasive population in integrated environment can assurance the emission of propagules to environments not yet invaded and cause colonization.

We conclude that higher temperatures contributed for that the initial phases had high densities, and that, this probably is related to increased source of food, as indicated by the increased biovolume the phytoplankton community. The phytoplankton community, independent of the size, contributed to increase *L. fortunei* larvae density because influence about adults of this specie.

The presences of adults in the determined locations indicate a larva already installed and is able to develop these new habitats. Therefore, to prevent the dispersion of these individuals it is necessary to understand the mechanisms of survival in basal levels of development. Understanding the process related to the development of larval stages would provide tools and information for conservation and management of aquatic environments, in order to of preventing the establishment of these individuals instead of manage the specie already established.

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