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Invasion by *Limnoperna fortunei* (Dunker, 1857) (Bivalvia, Mytilidae) of the Pantanal wetland, Brazil

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Abstract

Limnoperna fortunei (*Bivalvia, Mitylidae*) was introduced into South America in 1991 in the La Plata River (Argentina). It arrived in the ballast water of ships coming from Asia, where this species is native. It was first observed in 1998 in the Paraguay River. *Limnoperna* was introduced into the Pantanal region as hull fouling of vessels using the Paraguay–Parana waterway. This study describes how *L. fortunei* came to the Pantanal region, and provides details of its occurrence, density, and impacts. From 1999 to 2002, observations and sampling on natural and artificial substrates in the Paraguay River were made. Some aspects of the spread and impacts, based on local community information, were also analyzed. On artificial substrate the density reached 523.8 individuals m^{-2} and on natural substrate (rocks), up to 10,000 individuals m^{-2} were found. The densities observed were quite low compared to those found in Southern Brazil, where values up to 100,000 individuals m^{-2} have been recorded in the last 3 years. In the Paraguay River, the population density of *L. fortunei* can be negatively impacted by periodic low levels of dissolved oxygen and decreases in pH to between 5 and 6. Such conditions are frequently present during the periodic flooding or inundation of this area. Under these conditions, a high mortality of *L. fortunei* was recorded in March of 2002, on both natural and artificial substrates. Despite low densities, *L. fortunei* can colonize water cooling systems of boats, obstructing water circulation and causing motor overheating. Accumulation in water supply equipment, such as pumps and pipes has also been observed.

Introduction

The invasion of exotic species is a great threat to the integrity of aquatic ecosystems. The use of ballast water in large ships to confer stability and maneuverability has been an efficient means for the spread of marine and freshwater organisms. Around 12 billion tons of ballast water, containing approximately 4500 different aquatic species, are transferred around the world annually (Bashtannyy et al. 2001).

A freshwater species *Limnoperna fortunei* Dunker (1857), the golden mussel, was found in 1991 in brackish water of the La Plata River (Pastorino et al. 1993). It was probably introduced there via ballast water from ships that transport products from Asia to Argentina. *L. fortunei* is a native of China, occurring naturally also in Laos, Cambodia, Vietnam, Indonesia and Thailand. It has been unintentionally introduced into Hong Kong, Japan, Taiwan and South America in the last 30 years, where it has caused large-scale fouling in water supply systems and power plants, attaining densities of greater than 50,000 organisms/m^2 (Ricciardi 1998).

Limnoperna is a small mussel, about 3–4 cm in length, with several free-swimming life stages before attaching onto a hard substrate (Cataldo and Boltovskoy 2000; Darrigran 2002). It has adapted very well to freshwater environments in Argentina, and has affected the diversity of the native mollusk species and caused ecological changes in aquatic community structure (Darrigran et al. 1998; Mansur et al. 2003).

As a consequence of its fast reproductive rate, *L. fortunei* quickly forms extensive colonies with high population density. If colonies form on or in industrial water intakes, such 'biofouling' can result in clogged pipes and filters, changes in flow at the boundary layer, accumulation of empty shells, contamination of water from pipes due to mass mussel mortality, as well as disruptions in the water intake of industrial and power-generating plants. Operating costs increase due to lowered pump efficiency, tube corrosion as a result of enhanced bacterial and fungal proliferation, and service interruptions for the cleansing and changing of filters. These impacts are very similar to those caused by the zebra mussel (*Dreissena polymorpha* Pallas (1771)) in North America (Darrigran 2002).

L. fortunei has an enormous potential to spread. It is transported mainly as planktonic veligers within tanks, or as adults colonizing the hulls of vessels. As a result of the traffic of container ships between Argentina and Brazil, *L. fortunei* invaded the Paraguay River, reaching the Pantanal wetland in 1998 (Oliveira et al. 2000). The La Plata basin is the second largest watershed in South America. It interconnects five countries of South America, through the Paraguay–Parana waterway. The Paraguay and Parana Rivers, which form the La Plata basin, are utilized for commercial and tourist navigation, and have been the main avenue of the spread of *L. fortunei* into the center of Brazil.

In Brazil, the invasion of *L. fortunei* has also been documented in the Guaíba Lake basin, in the state of Rio Grande do Sul at the beginning of 1999. It was probably introduced there via ballast water (Mansur et al. 1999) and in the Itaipu reservoir in April of 2001 (Zanella and Marenda 2002), probably via boats used for sport fishing.

No information exists on the spatial distribution of *L. fortunei* in the Paraguay River basin, and studies on its biology and ecology in this new habitat are absent. The present study describes how *L. fortunei* came to the Pantanal region, and provides information on its range, population density and environmental impacts.

Materials and methods

Study area

The Pantanal floodplain is located in the upper Paraguay River basin, and has an area of about 140,000 km^2, mostly in Brazil. The Paraguay River runs from north to south collecting the waters of large tributaries on the left bank, including the Cuiabá, Taquari and Miranda Rivers. On the right bank, there is a series of extensive river-connected lakes, surrounded by higher land.

Flooding is seasonal and usually follows about 3 months after the rainfall in the north. Due to the time required to move down through the floodplain, the floodwater only reaches the study area between April and June, when rainfall has already stopped in the northern part. Flooding occurs as a result of river overflows, local precipitation or a combination of both processes (Calheiros and Ferreira 1997).

The direction of the water flow depends on the hydrological phase. It moves towards the river in the falling and dry phases and away from the river in the rising phase, before running again towards the river during the period of the full flood after the whole system coalesces.

The climate is hot, with mean annual temperature around 25 °C, oscillating between around 21 and 28 °C. Between the months of October and December, the absolute maximum air temperature exceeds 40 °C. The water temperature can reach 34 °C between October and March. The mean annual relative humidity is around 77%. Precipitation is most intense from November to March, with the mean annual accumulation being around 1070 mm, resulting in seasonal flooding (Soriano 1997). The lithology consists of alluvial sediments of the Pantanal formation, with loamy (clay) and sandy phases in alternate and discontinuous formations (Amaral Filho 1986).

Methods

Between 1999 and 2000, observations and samplings were made of *L. fortunei* in the Paraguay River from Forte Coimbra to Bela Vista do Norte, and in the lakes of Zé Dias and Castelo, as well as the Tamengo Channel. Mussels were observed on rocks, wood and bottom material. On rocks at the margin of the Paraguay River, in the vicinity of Bela Vista, the mussels were counted in 10 × 10 cm quadrants for density estimates (individuals m^{-2}).

Monitoring the density on natural substrate, such as rocks, is difficult because of fluctuations in water level along the course of the year. Fluctuations expose the substrate and cause mussel mortality. The waters are turbid and with low visibility, so that the use of artificial substrate is more suitable for studying the reproductive behavior.

From May of 2001 to August of 2002, the mussels were monitored on artificial substrates of concrete and wood fixed to an iron bridge on the Paraguay River, near Porto Esperança. The number of mussels was counted in quadrants of 0.09 m^2. In July of 2001, artificial concrete substrates were substituted for the wood substrate (X form, area 0.084 m^2). The first census was taken in August 2001 and repeated at monthly intervals between February and August of 2002.

To characterize the water of the Paraguay River, data for the years 2000 and 2002 from the locality of Porto Esperança were used. Subsurface water samples were taken with a Van Dorn bottle. Environmental parameters considered include: water temperature, dissolved oxygen and pH were measured with a portable potentiometer (YSI), calcium concentration was analyzed by atomic absorption (Perkin Elnas model 3300), turbidity with a turbidity meter, total nitrogen and total phosphorus were measured according to methods described in Wetzel and Likens (1991) and chlorophyll *a* was analyzed according to Marker et al. (1981).

Results and discussion

The route followed by *Limnoperna fortunei* after introduction into South America and the Pantanal, and its distribution in the upper Paraguay River Basin are represented in Figure 1. *L. fortunei* was first found in the Paraguay River in 1998. However, the size of the shells found (1.0–2.0 mm in length) suggests that the mussels were present for at least 1 year. This conclusion is based on the annual growth of *L. fortunei* in Japan, which has been estimated to be 15 mm (Magara et al. 2001). *L. fortunei* was observed for the first time in a bottom sample taken in a lake, connected to the Paraguay River, called 'baia do Tuiuiú' (18°49′18″ S and 57°39′13″ W), next to the city of Corumbá, in Mato Grosso do Sul State.

In January of 1999, in the Paraguay River in the vicinity of Forte Coimbra (19°53′34″ S and 57°46′44″ W), the exposed rocks due to very low water level, were observed to be covered by shells of *L. fortunei.* In the northern part of the Paraguay River, in the vicinity of Bela Vista do Norte (17°38′04″ S and 57°41′45″ W), extensive colonies of mussels on rocks were observed. *Limnoperna* has also been found in Castelo Lake (18°34′ S and 57°34′ W) and the Tamengo channel (10°59′ S and 57°40′ W). Occurrence has been observed in lotic, lentic and semi-lotic environments, at depths ranging from about 50 cm to 10 m. Colonization was also observed on submersed cages in aquaculture experiments, located in the Paraguay River. Mussels were attached to steel screens, nylon cords and plastic drums.

Density of *L. fortunei* on natural substrate in the Paraguay River was found to be about 10,000 individuals m^{-2} in September of 2002 (such values being restricted to small areas with rocks). This density is low compared to Guaiba Lake that had densities of 879 in 1999 and up to 22,856 individuals m^{-2} in 2000 (Mansur et al. 2003) and 100,000 individuals m^{-2} in January of

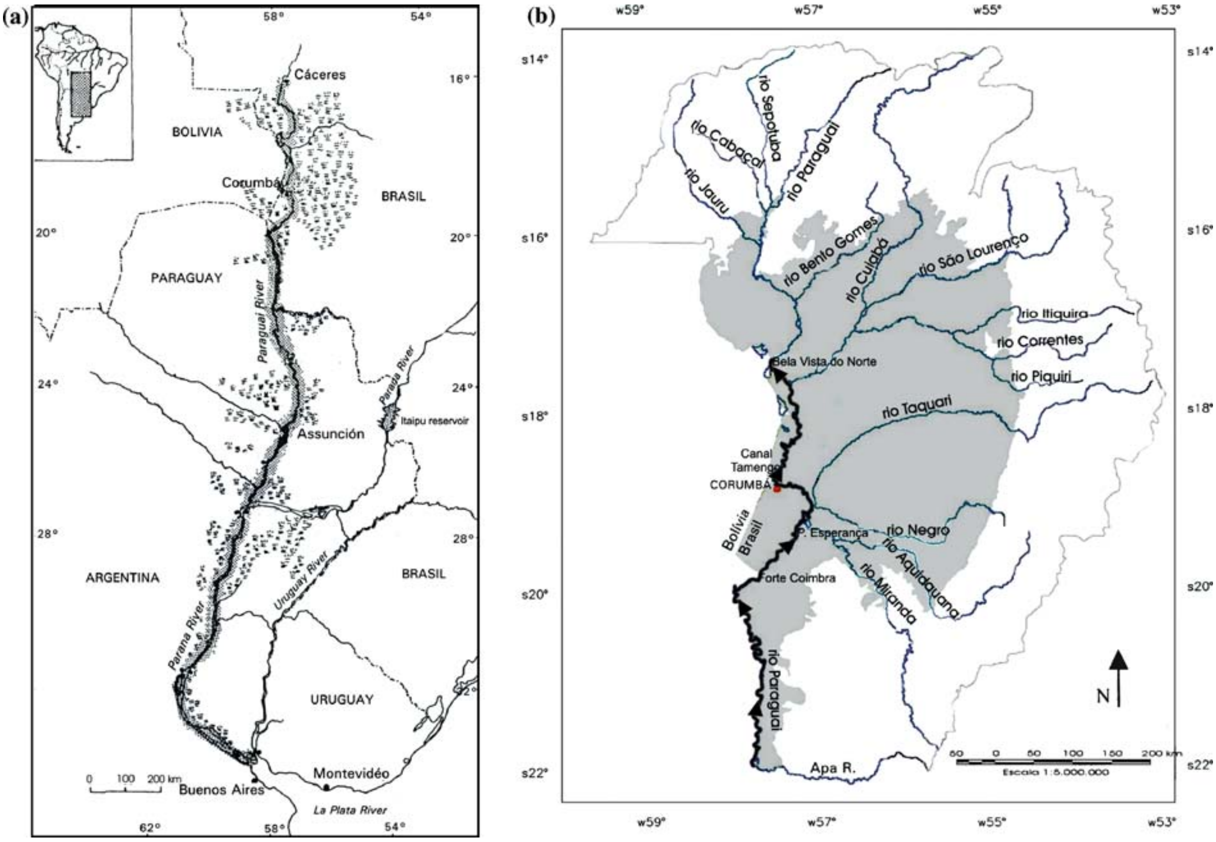

Figure 1. Spread of *Limnoperna fortunei* in South America along the Paraguay–Parana waterway and its distribution in the Paraguay River (dense black line). The gray area is subject to inundation.

2001. Similar density increases were observed in Argentina, with 4–5 individuals m^{-2} in 1991 and reaching 150,000 individuals m^{-2} in 1998 (Pastorino et al. 1993; Darrigran and Escurra de Drago 2000).

Mean density of *L. fortunei* on concrete substrate oscillated between 55.6 in May 2001 to 88.9 individuals m^2 in August 2001. Concrete substrate was not resistant to turbulence in Paraguay River. In 2001 this substrate was substituted by wood. On wood substrate the mean density was found to be 523.8 individuals m^2 in August 2001 (Table 1). The shell length of new settlers on both concrete and wood substrate varied from 3.0 to 10.0 mm.

In March 2002, the mussels present on the artificial or natural substrate died. Until August 2002, no new colonization on the artificial substrate was observed. This mortality occurred during the rising water phase (February and March of 2002). During this time, the concentration of dissolved oxygen decreased from 7.0 to about 0.3 mg l^{-1} for around 2 months (Figure 2). This is a natural phenomenon locally called 'dequada'.

The 'dequada' phenomenon is associated with organic matter decomposition in the beginning of

Table 1. Mean density of *Limnoperna fortunei* on concrete and wood artificial substrate in the Paraguay River, at Porto Esperança between May 2001 and March 2002.

Date	Substrate	Density (individuals m^{-2})
May 2001	concrete	55.6
July 2001	concrete	77.8
August 2001	concrete	88.9
	wood	523.8
February 2002	wood	238.0
March 2002	wood	0.0

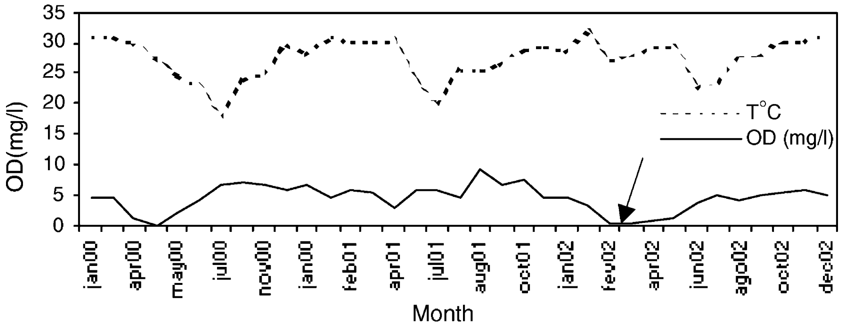

Figure 2. Dissolved oxygen and water temperature changes in the Paraguay River, between 2000 and 2002. The arrow shows a period when dissolved oxygen is close to 0.0 mg l^{-1}.

the flooding period, and causes great and rapid oscillations in water characteristics such as pH, conductivity, alkalinity, nutrient availability and especially gas concentrations (dissolved oxygen and carbon dioxide) (Figure 2). Depending on its magnitude, this phenomenon can cause mortality of up to a million tons of fish (Calheiros and Hamilton 1998). Effects on other components of the aquatic community have not yet been documented. This phenomenon occurs every year during the rising water phase, with intensity varying with the extent of flooding. Other Paraguay River water characteristics are shown in Table 2.

According to Claudi and Mackie (1994), water temperature, pH and Ca^{++} are the variables that most influence the development of *Dreissena polymorpha*, an invasive bivalve species in North America. In Argentina, water temperature is the main factor limiting *L. fortunei*, because reproductive activity has been found to cease at water temperatures below 13–17 °C (Cataldo and Boltovskoy 2000). According to Ricciardi (1998),

Table 2. Water characteristics of the Paraguay River, at Porto Esperança. Data are maximum and minimum values between March 2000 and December 2002.

Characteristic	Values (max–min)
Water temperature °C	17.9–31.8
Dissolved oxygen (mg l^{-1})	0.2–8.0
pH	5.7–7.4
Ca^{++} (mg l^{-1})	2.1–11.6
Turbidity (NTU)	3.7–68.3
Total nitrogen (mg l^{-1})	0.2–0.9
Total phosphorus (mg l^{-1})	N.D.–0.2
Chlorophyll *a* (μg l^{-1})	N.D.–17.0

N.D. – not detected.

who reviewed data on the tolerance of *L. fortunei* to environmental variables, the larval stage develops in temperatures between 11 and 33 °C, while the adults occur in temperatures from 8 to 35 °C. In the Paraguay River, fluctuating dissolved oxygen and pH are likely to be the primary control parameters. The water temperatures during the period of this study were below 17 °C, and probably did not limit reproductive activity. However, high temperatures, above 31 °C, are frequent in the Pantanal region between October and March, possibly reaching the upper thermal limit for adult *Limnoperna*. More study on the effects of such high temperatures on the larval and adult stages is required.

The ideal pH for infestations of *Limnoperna fortunei* is above 6.4 (Ricciardi 1998). Normally, pH of the water of the Paraguay River is higher than 6.5, but in the rising water period, the water can become more acidic (Table 2), thus probably contributing to the mortality of *L. fortunei* observed in March of 2002.

Adequate calcium concentration is fundamental to the invasion success of *L. fortunei*; values of above 3 mg/l are required (Ricciardi 1998). In the Pantanal, the calcium concentration was measured to be between 2 and 11.6 mg/l, with a mean of 4.6 mg/l (Table 2). Calcium is unlikely to be a limiting factor for *Limnoperna* development. All water parameters measured in the Paraguay River water are shown in Table 2, including nutrients (nitrogen and phosphorus) and chlorophyll *a*.

In the Paraguay River, annual water level fluctuations will periodically expose the mussels to air causing mortalities during the period of low water. Another factor, less important, is a paucity of substrate for colonization in Paraguay River because the bottom of the river is mainly sand, with few rocks. *Limnoperna* larvae need to find a substrate to settle on 15–20 days after hatching (Darrigran 2002). In the extension of Paraguay River there is a lot of submerged vegetation that could provide settlement substrate. If desperate, larvae can settle on individual grains of sand and then on each other as we observed in Paraguay River.

It is interesting to emphasize that two other mussel species of the genus *Corbicula*, introduced between 1996 and 1997, have lower density (192 individuals m^{-2}) in the Pantanal than in Southern Brazil (5191/m^{-2}) (Callil and Mansur 2002) and Argentina (2495 individuals m^{-2}) (Pastorino et al. 1993) suggesting that there exist limiting factors for mussel development in the Paraguay River.

In the Paraguay River tributaries, where the 'dequada' phenomena does not occur, *L. fortunei* might attain high densities, especially in the Miranda River. The waters of the latter are rich in calcium, with recorded values up to 38 mg/l (Leone 1990) and pH around 8. *L. fortunei* has been observed in the Miranda River since February of 2003 (Barros et al. 2003). This sub-basin presents one of the more diverse bivalve faunas of the Pantanal. Leone (1990) registered 14 species of native bivalves in four families. *L. fortunei* can encrust the shells of native mussels (Mansur et al. 2003). Bonito City, a tourist center where the rivers have crystalline water used for diving and other aquatic sports, is located in this sub-basin. In these waters, the aquatic plant biodiversity is very high. The introduction of *L. fortunei* in this location would be very undesirable, most likely causing severe environmental and economic impacts. The infestation in this area would be via small boats used for sports diving equipment or sport fishing.

Spread potential and impacts

Between 1991 and 1999, *L. fortunei* invaded freshwater ecosystems in five countries of South America (Argentina, Brazil, Paraguay, Uruguay and Bolivia) and, according to Darrigran and Escurra de Drago (2000), this species has advanced by 240 km/year in the La Plata basin.

Upstream dispersal is affected by navigation along the Paraguay-Parana system, with the adults of *L. fortunei* being transported adhering to the hulls of vessels and larvae inside tanks. Increments in these navigation would increase the invasive potential of *L. fortunei* as well as of other exotic species. Although *L. fortunei* has not been found in the northern part of the Paraguay River, the possibility of invasion exists due to the frequent navigation between the ports of Corumbá and Cáceres.

Downstream dispersal is effected by veliger from Paraguay River channel to connected-lakes with annual flooding or adults, incrusted on hull or tanks of small boats used for professional or sport fishing, that navigate between Paraguay River and tributaries. Probably this is the way that *L. fortunei* colonized connected lakes in the floodplain and two tributaries of Paraguay River until now.

The annual flooding could be another pathway of spreading *L. fortunei* in the Pantanal. According to McMahom (1982) and Darrigran (2002), larvae and juveniles can be transported on the feet and beaks of birds. Pathway of spread are also aquaculture related transfer of fish, bait that is taken from one river to another, sports diving equipment, fisherman nets and others.

Mussels are excellent filter feeders. They can accumulate heavy metals as well as other pollutants in their shells and flesh. Callil and Junk (1999, 2001) observed heavy metal accumulation in the tissue of native mussels, in the region of Poconé, MT. The heavy metal accumulated in the tissue can be transferred to fish. *Limnoperna fortunei* has been found to be used as food by fish belonging to the families *Characidae* (*Piaractus mesopotamicus*), *Anostomidae* (*Leporinus friderici*), *Pimelodidae* (*Pimelodus maculatus*) and *Doradidae* (*Oxydoras kneri* and *Pterodoras granulosus*) in the Upper Paraguay River, around latitude 17°51′49″ S and longitude 57°30′36″ W. The use of this mussel as food ranged from 12% in *Pimelodus macxulatus*, to 100% in *Piaractus mesopotamicus* and *Leporinus friderici* during June and September 2001. It is possible that with more intensive sampling in the Paraguay River, other fish families could be included in this list. Montalto et al. (1999) found *Limnoperna fortunei* in the stomach of several fish species of the family *Potamotrygonidae*, *Anostomidae*, *Doradidae*, *Pimelodidae* and *Loricariidae* in the Middle Parana River, at the confluence with the Paraguay River.

Mansur et al. (2003) found *L. fortunei* fixed on the shell and soft parts of native mussels, such as *Diplodon koseritzi* and *Leila blanivilliana* (Lea 1834), and the gastropod genus *Pomacea canaliculata* (Lamarck, 1822) in the Guaíba River (RS); these genera also occur in the Pantanal.

Despite the low density of mussels, economic impacts have been observed in the Pantanal. *L. fortunei* infested cooling systems of vessels, preventing proper circulation of water and leading to overheating and destruction of motors. Infestation has also been observed in water supply systems (pumps and pipes) and the water treatment station of a city in the region.

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