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# 8. Invasive species as a threat to biodiversity: The golden mussel *Limnoperna fortune* approaching the Amazon River basin

Marcela Uliano-Silva<sup>1</sup>, Flávio F. C. F. Fernandes<sup>2</sup>, Igor B. B. de Holanda<sup>3</sup> and Mauro F. Rebelo<sup>1</sup>

<sup>1</sup>Instituto de Biofísica Carlos Chagas Filho - Universidade Federal do Rio de Janeiro, 21941-902 Rio de Janeiro, Brazil; <sup>2</sup>Instituto de Estudos do Mar Almirante Paulo Moreira, 28930-000 Rio de Janeiro, Brazil, <sup>3</sup>Universidade Federal de Rondônia, 76808-659, Rondônia, Brazil

**Abstract.** *Limnoperna fortunei* (Dunker, 1758) is a freshwater bivalve that arrived in South America in the early 1990s. Today it is widespread in the La Plata, Uruguay and Paraguay river basins and is also present in the Tietê River and the Patos Lagoon system. The golden mussel has the capacity of fouling pipes, causing financial losses to hydroelectric power plants and water-supply companies. Further, this mussel is a very efficient ecosystem engineer, modifying physical and biotic elements in the ecosystems where it occurs. Its high tolerance to different environments together with human assistance through ballast-water discharge and transport on boat hulls posses a high possibility of its introduction into Amazonian waters. In an attempt to respond to this risk, some measures have been taken; among these, the discharge of chloride into the water has been ineffective in killing mussels and is jeopardizing other aquatic organisms. The Brazilian federal government and the Navy are taking precautions against its spread, and Brazilian researchers are studying

the biology of the golden mussel in order to find a way to kill it without harming the environment. However, the situation is not yet totally under control; education might be the only way to prevent the golden mussel from dispersing northward from the city of Cáceres in the Brazilian Pantanal wetland, the present northern limit of its distribution.

#### 1. Limnoperna fortunei

Limnoperna fortunei (Dunker, 1758) is an Asian bivalve mollusk that arrived in South America in the early 1990s. Twenty years later, it is a major plague in the southern waters of this continent and is spreading rapidly. The first occurrence of this species in South America is well documented by Argentine and Brazilian researchers [1, 2, 3, 4], but most of the information regarding its relationship to the environment has been published in local journals or proceedings of regional conferences, which makes it difficult to pull together reliable information.

#### 2. Mussel dispersal in South America

In 1991, *L. fortunei* was found for the first time in the estuary of the La Plata River [1]. In the beginning of 1994, it was described also on the Uruguayan coast, at the beach resort of Artilleros [5]. Darrigan & Pastorino [6] suggested that *L. fortunei* was introduced into South America through ballast water from ships coming from Hong Kong or Korea. Between 1991 and 2000, *L. fortunei* had already arrived in the three major rivers of the La Plata basin: Paraná, Paraguay and Prata (Figure 1) [7].

Its success as an invader is related to its habits and biology. L. fortunei shares many biological characteristics with the zebra mussel Dreissena polymorpha, which is causing extensive economic and ecological problems in North America [8]. Both species attach to any solid substratum through their strong byssus, and grow rapidly, reaching sexual maturity by the age of 1 year. They have short life spans (2-3 years) and possess a planktonic (veliger) larval stage [9, 10, 11]. The number of byssal fibers secreted by L. fortunei (up to 100 or more) is related to the amount of strength it needs to attach on the different surfaces [12, 13]. L. fortunei is dioecious, with external fertilization, and reproduces at least once a year. It is capable of inhabiting waters with relatively low calcium ( $\geq 3.0$  mg/L) and pH ( $\geq 6.4$ ) levels and can tolerate a wide range of water temperatures (8-35 °C), which enhances its ability to colonize a wide range of different environments [14]. This species has the potential to form dense aggregates. In 1991, there were 5 organisms.m<sup>-2</sup> on rocks of the La Plata River at Bagliardi in Argentina. The density then increased to 30,000 organisms.m<sup>-2</sup> in 1992,

82,000 organisms.m<sup>-2</sup> in 1993, and reached an astonishing 150,000 organisms.m<sup>-2</sup> in 2002 [7].

The golden-mussel invasion in Brazil occurred at practically the same time in two different locations and pathways. First in 1998, in the Paraná River, in Mato Grosso do Sul in midwestern Brazil [4], through the migration of specimens from the La Plata River. The mussel population started to move up the Paraná and Paraguay rivers, assisted by the heavy ship traffic. In the same year, it was introduced into the Jacuí River near Porto Alegre, southern Brazil [15] probably through ballast water (Figure 2B). In 2000, it was already present in the Patos Lagoon and Guaíba River. Today it is present in rivers, lagoons and water reservoirs in Mato Grosso do Sul, São Paulo and in southern Brazil (Figures 1 and 2).

In 2001, *L. fortunei* was detected at one of the largest hydropower plants in the world, the Itaipu power plant (Figure 2B). More than 80% of Brazilian electricity comes from hydroelectric sources. With an 8-km-long dam and a production of 97,000,000 MW/year, Itaipu dams the Paraná River which is the 10<sup>th</sup> largest river in terms of water volume in the world and a major water resource for three countries, Brazil, Paraguay and Argentina. The Paraná River is one of the main tributaries of the La Plata River, where the invasion took place more than 20 years ago. When the golden mussel was first detected in the Yacereta power plant on the border of Paraguay and Argentina, in 1998 [6], technicians from the upstream Itaipu became concerned and established a monitoring program for larvae and adults in the river downstream from the dam. To their surprise, they found the first *L. fortunei* attached to the steel grate protecting the raw water intake at the lake, upstream from the dam, in 2001 [16]. The enemy came from behind.

Since its first appearance in South America, scientists have calculated the dispersal speed of *L. fortunei* to be 240 km.year<sup>-1</sup> upstream [17]. It is very unlikely that this rapid rate is achieved only by larvae dispersal against the currents of these very large rivers. There must be an alternative way for the golden mussel to move upstream, but what is it?

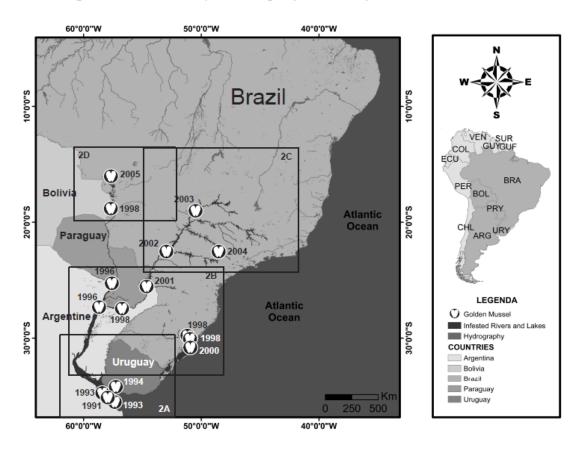
Small fishing boats and canoes are widely used as transport for sport fishing in Brazil year-round. Fishermen move from one river to another looking for the best catch, and transport their boats by motorized vehicles. Technicians from the Brazilian Institute of Environment and Natural Resources (IBAMA) eventually realized that mussels were hitchhiking in or on the hulls of these small boats. Although poorly documented, this remains the main form of dispersal for *L. fortunei* in Brazil.

Transport by boat is probably the route by which mussels moved up the Paraguay River to arrive in Cuiaba, in the Brazilian Pantanal wetland in 1998 [18] as well as in the Paraná and Tietê rivers (Figures 1, 2C and 2D). In some

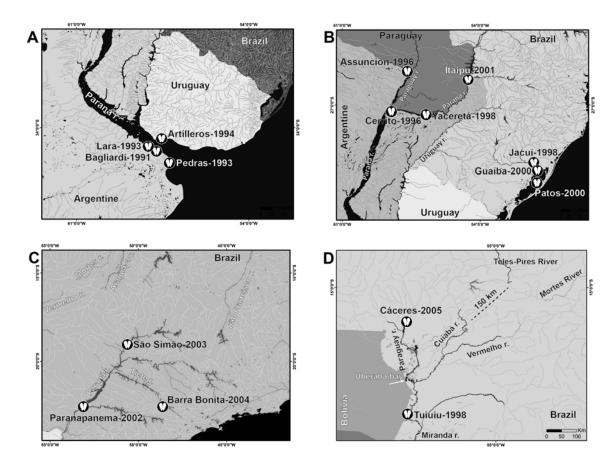
of these watersheds, the productive fisheries support annual international fishing tournaments, such as at Cáceres in western Brazil. Involving more than 45 cities along four rivers, during 15 days with hundreds of boats from all over the world, this is the largest Latin American fishing tournament. The Miranda River (Figure 2D) is the second-largest fish producer in Mato Grosso do Sul, after the Paraguay River, and it is clear to the Brazilian authorities that the golden mussel was introduced there mainly by boats towed across the main highway BR 262, from one river to another during fishing festivals in 2003.

#### 2.1. How to control this mussel dispersal?

Considering the continental dimensions of a country such as Brazil, we might as well say that it is not possible. Education may be the only realistic alternative to mitigate this risk. Accordingly, the Brazilian electrical company Furnas implemented a major campaign among riverine communities and



**Figure 1.** The distribution of *Limnoperna fortunei* in South America. The year of the first record at each location is shown. The invasion started in Argentina in the La Plata (Paraná) River, and moved northward to colonize the entire river basin and at least three other main river basins: Uruguay, Paraguay and Tietê rivers, as well as the Patos Lagoon system in an independent invasion event. The present northern limit of distribution is at the city of Cáceres in the Brazilian Pantanal.



**Figure 2.** Detailed locations of *Limnoperna fortunei* in South America. Detail A: the first occurrence was recorded at the coastal resort of Bagliardi in 1991 (34°55' S - 57°49'W). It then spread south to Punta Pedras in 1993 (35°26'S - 57°08'W) and north to Punta Lara in 1993 (34°48'S - 57°59'W). In 1994 it reached the town of Artilleros (34°27'S - 57°32'W) on the Uruguayan bank of the La Plata River. Detail B: in 1996 the mussel was present at Cerrito Island (27°20' S - 58°43'W) and in the Asunción Harbor (25°17'S - 57°38'W) in the Paraguay River. In 1998 it also was present at the Yaceretá Hydroelectric Power Plant (HPP) (27°29'S - 56°44'W), Tuiuiú Bay in 1998 (18°49'S - 57°39'W), Jacuí Delta in 1998 (30°02'S - 51°13'W), Guaíba Lake in 2000, Patos Lagoon in 2000, and Itaipu HPP in 2001 (24°05' and 25°33'S - 54°00' and 54°37'W). Detail C: *L. fortunei* present in the Paranapanema River in 2002 (22°31'S - 53°00'W), São Simão HPP in 2003 (20°34' and 20°37'S - 41°28' and 41°30'W), and Barra Bonita HPP (Tietê River) in 2004 (22°29' 59"S - 48°34'17"W). Detail D: The present northern limit of distribution at Cáceres City (2005). From Cuiabá River, the golden mussel it must travel only 150 km to reach the Téles Pires River, which has connections with Amazon basin rivers.

schools along the main Pantanal rivers. With the slogan "Don't let the golden mussel hitchhike", the Furnas-sponsored educators have visited many communities since 2003 (see the website of the campaign http://www.furnas.com.br/frmMAAcoesMexilhaoDourado.aspx - Portuguese only), advising people about the importance of disinfecting a boat's hull before putting it in the water.

### 3. The 'dequada' phenomenon in the wetlands helping to contain golden mussel dispersal

The northern limit of *L. fortunei* distribution is the city of Cáceres in the Pantanal watershed (Figure 2D). The question arises, why the golden mussel is not moving farther north from there? Researchers working in that area realized that the environmental phenomenon called 'dequada' is the likely reason why a permanent mussel population has not become established there [19, 20].

As a part of the yearly flood cycle of the Pantanal, when the water level recedes to 10% of the maximum volume, the aquatic vegetation dies and is replaced by terrestrial flora (mainly grasses). When the water rises, the submerged terrestrial vegetation is killed and to decompose, together with the remains of the aquatic plants. This phenomenon causes wide and rapid oscillations in water characteristics including pH, electrical conductivity, alkalinity, nutrient availability and especially gas concentrations. During this period, the water can become anoxic and CO<sub>2</sub> can reach up to 100 mg.L<sup>-1</sup>. Water temperature can rise to 32°C, contributing to organic-matter decomposition and water-quality deterioration. This can cause extensive fish mortality [12] and, fortunately, also high golden-mussel mortality.

Which parameters involved in the 'dequada' might account for the golden mussel decline? We do not know. It is difficult to identify such a marker for a species such as *L. fortunei*, which has a wide tolerance of ecological parameters, varying according to its geographical location. Moreover, the 'dequada' is a complex phenomenon that might be governed by several forces, none of which might directly control mussels in the Pantanal. Even if a single controlling factor exists, it might not even matter: even if we find it, how could we control it in the natural habitat?

### 4. Exotic in the wild: The ecological impacts caused by L. fortunei

Because *L. fortunei* shares several ecological traits with the zebra mussel (*D. polymorpha*), scientists have predicted that they will also have similar impacts on the ecosystem [21]. *D. polymorpha* is considered the most aggressive freshwater invader in the northern hemisphere. Originally from the Caspian and Black seas of Europe, it was first reported in Lake St. Clair in the United States in 1988. Today it is present in all of the Great Lakes and in other inland rivers and lakes in the American midwest [22]. Extensive research has shown its ecological impacts: it changes existing habitats and provides new habitat for other organisms, affects trophic interactions and the availability of food for pelagic and benthic species, and influences the rates of other processes such as

mineralization of nutrients, oxygen availability, sedimentation rates, and dynamics of pollutants (for reviews see 21, 22, 23, 24).

Although the ecological impacts caused by *L. fortunei* in South America are not as well studied as those caused by *D. polymorpha* in Europe and North America, some evidence of environmental modifications is available. Darrigran [8] showed that *L. fortunei* was associated with increases in the density of isopods, amphipods, oligochaetes, chironomids and turbellarians. The golden mussel colonizes crustacean carapaces and valves of unionid clams such as *Anodontites trapezeus* [15]. The attached mussels can prevent their hosts from opening their valves for respiration, feeding and reproduction, eventually killing the clams (Figure 3)[8, 15]. The high filtration rates (200-300 ml.h<sup>-1</sup>) of *D. polymorpha* and *L. fortunei* have similar effects on the abiotic parameters of aquatic systems: increased water transparency and light penetration, decreased concentrations of seston and organic matter, and increased ammonia, nitrate and phosphate concentrations [8].

Fish communities can also change. *L. fortunei* provides a novel resource available on an unprecedented scale: omnivorous fish that previously consumed a variety of prey are now feeding exclusively on *L. fortunei*. The Armado (*Oxydoras kneri*) and Pacu (*Prochilodus variegatus*) are two such species. In the La Plata River, up to 100% of the fish caught in the summer have their guts filled with remains of this mollusk [25, 26, 27] (Figure 4). The impacts on these fish communities are not limited to species that consume the mollusk directly; larger fishes such as *Pseudoplatystoma fasciatum* and *Salminus maxillosus* can benefit indirectly, as they feed on smaller fish that



**Figure 3.** Unionidae infested with *L. fortunei*. The infestation prevents the clams from opening their valves for respiration, feeding and reproduction. (Picture by Pr. Dr. TomazVitallAguzzoli).



**Figure 4.** The sequence of images shows the dissection of the 'Armado' *Ptedoras granulosus* collected in 2005 at Itaipu Hydroelectric Power Plant reservoir. The animal is clearly disformed and the reason is shown in the subsequent pictures, intestines filled with hundreds of (even alive) *L. fortunei*. (Picture by Prof. Dr. Carlos Eduardo Belz – Center for Marine studies, Federal University of Paraná).

consume *L. fortunei* [17]. Boltovskoy [17] suggested that a 2.4-fold increase in landings of freshwater fish between 1992-1993 and 2000-2001 was associated with the introduction of *L. fortunei* in Argentinean freshwaters.

Almost no information is available with respect to parasites and commensals associated with *L. fortunei*. However, Ogawa [28] identified widespread parasitic infections by bucephalid trematodes in cyprinid fishes from the Uji River, Japan. The author suggested that the infestation started with the accidental introduction of infested first intermediate hosts, *L. fortunei*.

## 5. Amazonia at risk: Invasive *L. fortunei* threatening the highest biodiversity in the world

Darrigran & Damborenea [29] observed that *L. fortunei* is not only an aggressive invasive species, but also a very effective ecosystem engineer. What would be the consequences if it arrives in the Amazon basin, a 7 million km² region almost as large as the continental United States? This basin includes territories belonging to nine different nations: Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela, Guyana, Suriname and French Guiana, and contains over half of all remaining tropical forests in the world.

In a study to predict the expansion of the golden mussel in Brazilian rivers, Oliveria et al. [30] concluded that locations in the Amazon basin, including the upper section of southern Amazon tributaries such as the Tapajós, Teles Pires and Araguaia rivers, are suitable for the occurrence of *L. fortunei* in terms of limnological conditions. The history of the invasion shows that *L. fortunei* was introduced into waters with low salinity levels. Therefore, ports located in fresh waters, such as Manaus and Belém harbors, must remain alert.

The Paraná River basin, where the golden mussel is now widespread, borders several other important river basins in Brazil, especially the São Francisco, Tocantins and Amazon (Figure 2D). The proximity of these basins indicates how easily the golden mussel could reach Amazon waters. For about 1,480 km, the Téles Pires River runs from Mato Grosso to the confluence with the Tapajós River, at the border of Pará state (Figure 2D). The Tapajós River belongs to the Amazon River basin, and if the golden mussel reaches its border it is only a matter of time until it becomes established there.

#### 6. Fouling of water-supply systems

The entrainment of planktonic larvae in intake currents allows *L. fortunei* to invade municipal and industrial water-supply systems rapidly. Although strainers and screens may be able to filter out adult mussels, the larvae pass through. Once inside pipelines and conduits, the golden mussel initially colonizes crevices, seams and joints, and then spreads from these foci to cover adjacent surfaces with clusters of byssal-attached mussels [9]. The attachment and dense accumulation of shells reduces flow through narrow pipelines that need expensive cleaning, increasing the number and duration of plant shutdown periods and therefore operational costs.

In 1995, Darrigran [31] recorded the first macrofouling of this bivalve in the water-intake system of the drinking-water supply company of La Plata city. Other, similar events were reported in the same year, interrupting the water intake for the Bernal and Buenos Aires municipal water-supply systems [2, 31].

However, in most of the world including Brazil, the largest economic impacts due to the presence of an exotic species are found in the electrical-power sector (O'Neil, 1997). A 120 MW hydroelectric power plant with the macrofouling problem in its cooling system may incur a daily loss of US\$ 20,000 [32]. At the Argentinean/Paraguayan Yaceretá hydroelectric power plant, fouling by *L. fortunei* has forced its turbines to shut down for cleaning several times since 1998 [6].

In Brazil, the largest hydroelectric exploitation potential (106,000 MW) is in the Amazon River basin, where the Tucuruí (4.245 MW) and Balbina (275 MW) hydroelectric power plants are located; and where the Brazilian government will be building many others in the next few years [33]. Therefore, a golden-mussel invasion in the Amazon will jeopardize not only the biodiversity, but one of the main sources of electrical-power production in Brazil.

The United States spends around 120 billion dollars every year dealing with damages caused by exotic species, including 1 billion dollars to control the zebra-mussel invasion [23, 32, 34]. This problem has so far remained unsolved. In South America, we still have time to prevent it; but if we fail to implement control actions, economic as well as ecological losses caused by *L. fortunei* [12, 27] may be catastrophic.

### 7. An alternate attempt: The use of toxicants to control infestation

Since we cannot rely on natural factors alone to prevent the mussel infestation, methods for chemical control have been developed and applied. Unfortunately, no presently existing method can be considered definitive. Many substances have proved to kill *L. fortunei*; however, often we are not even able to determine their mechanism of action, and more broadly, we certainly do not understand the effects of these substances on the environment.

The most widespread substance used in mussel control is chlorine, mainly because it is very cheap and is already regulated by law. However, its efficiency in killing mussels is actually very low, whereas the risk to industrial-plant installations and the environment is very high. De Kock & Bowner [35] showed that bivalves can sense many toxicants, including chlorine, dissolved in water, and can close their valves and remain closed for long periods. In the water, chlorine stimulates bivalves to close their shells, which eventually causes them to asphyxiate or starve to death. In order to kill adult mussels, chlorine must circulate in the system in high concentrations for long periods, and otherwise it is ineffective. However, it is not ineffective in the environment: chlorine can decompose to halogen compounds, and its toxicity to humans and the environment has been thoroughly reviewed [36].

Taking into account that bivalves feed on particulate matter, a more effective strategy could be to deliver the chemicals in that form rather than dissolved in water. A microencapsulated poison has been created by an English group to combat *D. polymorpha* [37]. Calazans [38] tested "biobullets," a commercial blend of microencapsulated KCl and quaternary ammonia in *L.* 

fortunei, with promising results. The LC50<sup>1</sup> for microencapsulated KCl for *L. fortunei* is 10 times lower than in the dissolved form. The same study demonstrated similar effects for other microencapsulated substances. Since the amount of chemical released into the environment in microencapsulated form is substantially lower, it may be a more environmentally friendly alternative to treat *L. fortunei* fouling.

### 8. Paint it black: The use of anti-fouling ink to prevent dispersal

A variety of chemicals have been tested by different groups [39, 40] in order to evaluate their efficiency as antifouling agents. However, in many cases the safety of these compounds in the environment has not been investigated.

Since 2008, TBT-based inks have been banned because of their high toxicity [41]. By inhibiting the function of mitochondria, TBT negatively affects the growth, development, reproduction and survival of many marine species [42, 43]. However, its organic substitutes, such as isothiazolone and zinc pyrithione, also have drawbacks. Of particular concern is the synergistic interactions between these biocides, which enhance their toxic effects [44]. CuO<sub>2</sub> is the substance most often used in contemporary antifouling inks. The general acceptance of copper is based on its widespread occurrence in nature and that it is essential for the development and normal growth of most plant and animal species [44]. Nevertheless, the effect of copper depends on the dose, and in higher doses, copper is as toxic as any of its predecessors.

### 9. Molecular biology in the fight against L. fortunei infestation and dispersal

In order to defeat *L. fortunei*, it is indispensable to have a thorough understanding of its biology. This includes knowing the molecular mechanisms involved in reproduction, growth, attachment to substrates, and resistance to stress, i.e., the characteristics that determine the golden mussel's success as an invader.

For this reason, our research group (BioMA Laboratory, part of the Biophysics Institute of the Federal University of Rio de Janeiro, Brazil) is currently sequencing the golden mussel transcriptome (expressed genes). The next-generation sequencing technologies that we are employing (GS Jr System –

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<sup>&</sup>lt;sup>1</sup> Concentration needed to kill 50% of the population

Roche, 454) allow us to rapidly characterize the genetic constitution of *L. fortunei* and then search for specific genes involved in its high acclimatation capacity of colonizing a wide range of different environments. At present, 95 thousand nucleotide sequences and around 7 thousand genes have been mapped for this species, and bioinformatics analyses are continuing. This process will eventually locate specific genetic determinants of metabolism that will allow us to investigate golden-mussel biology much more deeply. This line of investigation substantially increases the possibility of developing more-effective and less environmentally harmful strategies for this battle against the golden-mussel invasion.

#### 10. A persistent threat: Ballast water

Since *L. fortunei* was apparently able to survive trans-Pacific voyages in ballast water to arrive in South America, shorter trips within one continent or country are highly possible. Again, this poses one of the main threats for an invasion of the Amazon. Brazil has more than 7,000 km of coastline and most shipping traffic is local or regional. Although most routes involve one or more sea legs, many of these smaller vessels dock in freshwater harbors. Therefore, the risk is high that these vessels may transport *L. fortunei* from infested to uninfested harbors. Ballast water is the most certain dispersal route for the golden mussel across river basins.

The Global Ballast Water Management Programme (GloBallast) is an initiative of the International Maritime Organization (IMO) together with the United Nations Development Programme (UNDP) and the Global Environment Facility (GEF) to assist developing countries to reduce the transfer of aquatic exotic species through ballast water. In 2002, they launched the Golden Mussel Project in Brazil (Portaria No. 494, 22 December 2003 – <a href="https://www.furnas.com.br">www.furnas.com.br</a>). Supervised by the Brazilian Ministry of the Environment (MMA), coordinated by the Admiral Paulo Moreira Marine Research Institute (IEAPM) of the Brazilian Navy, the Golden Mussel project was an attempt to educate Brazilian governmental agencies and industry on how to control this risk situation. The NORMAM 20 law was a result of this initiative. It requires a series of actions from vessels navigating in Brazilian waters, including completion of the *ballast water reporting form*, stating where and when their tanks have been deballasted. Based on data from that study, Brazilian authorities have mapped the most vulnerable harbors in the country.

However, these forms by themselves cannot prevent invasions, since they are analyzed by the Admiral Paulo Moreira Marine Research Institute (IEAPM) after deballasting has taken place. In 2007 one of these forms reported a ship from Porto Alegre, a highly infested port, discharging its ballast

water in a port in the heart of the Amazon River. Fortunately, this single event was not sufficient to initiate an effective settlement, but this incident shows that the risk of introduction is real and it may be only a matter of time until a population becomes established.

The NORMAM 20 law also requires ballast water to be exchanged 200 miles away from the destination harbor, and a second deballasting at the mouth of the Amazon River. All these procedures were implemented in order to prevent *L. fortunei* from dispersing to new locations. However, the question remains: will these measures be sufficient?

#### 11. The present risk of introduction: Conclusions

Since its first appearance in 1991 in the La Plata River, *L. fortunei* has colonized every river and estuary that it could reach, eventually establishing in the waters of the Pantanal in the heart of the continent. Its high tolerance to different environments, together with human assistance through ballast-water discharge and transport on boat hulls, have made this situation very complex and dangerous. However, we cannot give up.

It may be naïve to presume that we can prevent the dispersal of *L. fortunei*, but it is also frivolous to assume that these efforts will eventually be in vain. The expansion of infestation is not inevitable; every year of successful prevention is one more year of lessons learned on how to control it. The molecular biologists are at this moment studying the golden-mussel genetics in order to better understand it and find a way to end this infestation. Brazilian authorities are educating the general public in how to avoid giving rides to mussels on their boats, the navy is making efforts to supervise the vessels and stop distribution through ballast water, and chemicals and antifouling inks are available to help to contain it.

There is no other option but to continue tirelessly with all possible actions in operation, including scientific research. That is the only reasonable strategy to fight this pest.

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