

Golden mussel

Limnoperna fortunei

**National Prevention, Control
and Monitoring Plan in Brazil**

Ministry of Environment

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Presentation

The golden mussel (*Limnoperna fortunei*) is a species of introductory bivalve mollusc introduced in Brazil via ballast water in the 1990s. Given its biological and ecological characteristics, as well as the favorable environment in the country for its proliferation, the golden mussel has become an invasive alien species. The biological invasion of this species has caused environmental and economic impacts, causing structural and functional changes in ecosystems and harm to human activities in the South, Southeast, Central-West and, lastly, in the Northeast regions, due to its recent detection in the São Francisco River Basin.

The Federal Government established in its Multi-Year Plan (PPA 2016-2019) the goal to control three invasive alien species, mitigating the impact on Brazilian biodiversity. The implementation of the goal includes the development and implementation of control plans for the prevention, early detection, eradication and monitoring of invasive alien species. In this sense, the Ministry of the Environment, together with its affiliates (Ibama and ICMBio), is working on the development and implementation of national plans for the prevention, control and monitoring of invasive alien species. For the first stage, the golden mussel (*Limnoperna fortunei*), the wild boar (*Your scrofa*) and the sun coral (*Tubastraea* spp.) were defined as priority species. The Wild Boar Plan was prepared in 2016 and published by Interministerial Ordinance No. 232, of June 23, 2017. The Coral-sol Plan is in the consolidation phase for publication.

Aiming to continue a series of actions for the National Task Force, by part of the MMA, and a series of other actors in society involved with the issue, the National Plan for Prevention, Control and Monitoring of the Golden Mussel in Brazil was drawn up (*Limnoperna fortunei*) – Golden Mussel Plan, firstly by preparing a diagnosis, compiling information on the invasion of the bivalve in Brazil and in neighboring countries, where the species is present. In a second stage, a public consultation was carried out on this diagnosis, with the objective of complementing the information and identifying weaknesses. Finally, a workshop was held for the participatory preparation of the Golden Mussel Plan, involving various actors from society.

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PART I

DIAGNOSIS

1. Biology and Ecology

1.1 Taxonomy

The species *Limnoperna fortunei* (Dunker, 1857), popularly known in Brazil as golden mussel and internationally as *golden mussel*, is one of two species of freshwater mussels in the family Mytilidae, which is mostly represented by mussels that inhabit oceans and estuaries. The golden mussel has an original distribution restricted to China, but has expanded to other countries in Asia and to South America (item 2). The systematic classification of *L. fortunei* follows as per Table 1.

Table 1. Systematic framing of *Limnoperna fortunei*.

Taxonomic Categories	Classification
Kingdom:	Animalia
Phylum:	Mollusca
Class:	Bivalvia Linnaeus, 1758
Order:	Mytiloida Ferrusac, 1822
Family:	Mytilidae Rafinesque, 1815
Gender:	<i>Limnoperna</i> Rochebrune, 1882
Species	<i>Limnoperna fortunei</i> (Dunker, 1857)

Until the 2010s, taxonomic studies indicated that the genus *Limnoperna* Rochebrune, 1882, comprised nine species, being *L. fortunei* (Dunker, 1857), the only representative of the genus and family Mytilidae in freshwater and the remaining species exclusively estuarine. Current review of the complex of species classified in the genus *Limnoperna* carried out by Colgan and Costa (2013), based on DNA sequences, concluded that estuarine species comprised a distinct complex, attributed to the genus *Xenostrobus* Wilson, 1967, including estuarine invasive species such as *Xenostrobus securis* Lamarck, 1819.

Considering the advances mentioned in the taxonomy, *L. fortunei* was confirmed as the only species of the genus in freshwater, but not the only Mytilidae. In a sympatry relationship with *L. fortunei* in Cambodia and occupying the same habitats, a species was described from the Mekong River, initially recognized as Dreissenidae, *Dreissena harmandi*. In review

taxonomic analysis carried out by Morton and Dinesen (2010), based on anatomical characters, it was concluded that the bivalve previously classified in Dreissenidae was in fact a Mytilidae. In addition, a reevaluation of the genus was carried out. *Sinomytilus*, to which the species previously described for the genus was transferred *Dreissena*. That way, *Sinomytilus harmandi* (Rochebrune, 1881) became the second Mytilidae that lives in fresh water, together with *L. fortunei*. To date, no introductions of *S. harmandi* outside its original area of distribution.

The golden mussel differs from the invasive species of the family Dreissenidae, mussels zebra finch (*Dreissena polymorpha*) and guaga mussel (*Dreissena rostriformis bugensis*), due to the absence of a septum, an internal structure typical of the umbonal region of the Dreissenidae shell.

1.2 Invasive Species Attributes

Considering the criteria established by the Convention on Biological Diversity, According to logic, the golden mussel is classified as an Invasive Alien Species, since its introduction and/or dispersion threatens biological diversity. Invasive alien species are those that, once introduced from other environments, adapt and begin to reproduce and proliferate, often causing changes in natural ecological processes, harming native species, habitats and ecosystems. In addition, invasive alien species can pose a risk or negatively impact society or the economy (MMA, 2016). According to Darrigran and Damborenea (2011), *L. fortunei* It is considered an engineering species, due to the potential structural changes it can cause, based on its filtration and macroagglomeration capacity.

Boltovskoy (2015) highlights that the success of the invasion and dispersal of the golden mussel The occurrence in South America is due to five key factors, two of which are attributes of the bivalve: (1) the free-swimming larva, which facilitates the dispersal of the mollusk in the plankton; and (2) a sessile and byssate adult, which occupies consolidated substrates, including ship hulls, creating encrustations that can be transported over long distances. The other factors cited by Boltovskoy (2015) are related to human civilization and constitute introduction pathways and vectors of dispersal in intercontinental and continental environments: (3) increased interconnectivity between basins via waterways, land-based vessel transport, basin transposition; (4) construction of reservoirs; and (5) growth of navigation in continental waters. These factors that influence the invasibility of areas geographically distinct from the original distribution area of the golden mussel will be discussed throughout the document.

Other attributes of the invader can also be cited as relevant factors for the success of the invasion in the South American continent: almost continuous reproduction, during an annual cycle (BOLTOVSKOY et al., 2015a), tolerance to a wide pH range (PEREIRA, 2014), tolerance to low calcium concentration (OLIVEIRA et al., 2010a), tolerance to desiccation

(DARRIGRAN et al., 2004) and survival during passage through the digestive tract of some fish species (BELZ, 2009).

1.3 Morphology

1.3.1 Larva

The external morphology of the larval stages of the golden mussel is described in Santos et al. (2005). Mansur et al. (2012) present a dichotomous key to differentiate the larvae of other native and invasive limnic molluscs from the larvae of *L. fortunei*. A summarized description of the veliconcha larva (Figure 1), presented by Mansur et al. (2012), is as follows: it presents a straight hinge, without central deflection and without projections at the terminal ends; the external surface of the valves is subdivided into prodissoconcha I, occupying approximately 2/3 of the height, followed by prodissoconcha II, which occupies the remaining 1/3. Prodossoconcha I is formed in the larval stage "D", only by the production of the shell gland that leaves a demarcation in the form of small holes in the central region close to the hinge. When the larval mantle is complete, the shell is produced only by the edges of the mantle, acquiring the striated comarginal demarcations that accompany the growth of the shell and form prodossoconcha II. When the veliconcha is complete, we have the larva in the veliger stage, which surrounds the entire body, protecting it when closed. When it is half open, it exposes the veil, a ciliated organ responsible for the animal's locomotion. The larva's movements are generally circular and vertical.

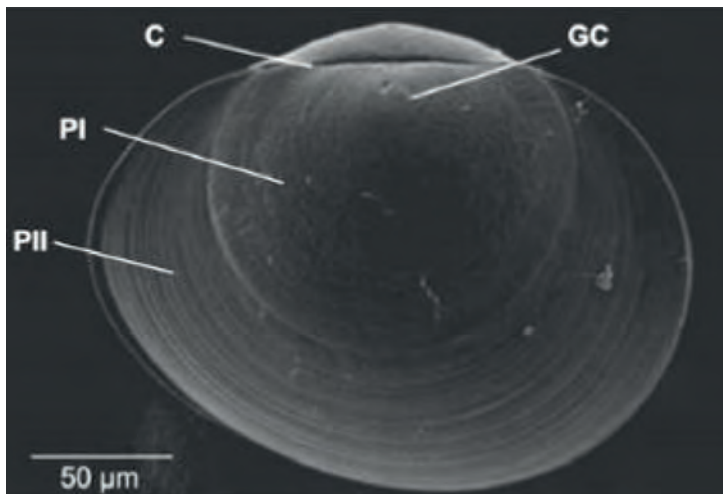


Figure 1. Side view of the veliconcha *Limnoperna fortunei*, under scanning electron microscopy. Hinge (C); demarcation of the shell gland (GC); prodossoconch I (PI); prodossoconch II (PII). (Preparation and photo: G. Figueiredo and MCD Mansur). Source: Mansur et al. (2012).

1.3.2 Adult

Aspects of the internal and external morphology of adult individuals of *L. fortunei* are described in detail in Mansur et al. (2012) and Morton (2015a). Like other bivalves, the golden mussel has two valves, which have a triangular, modioliform outline.¹(MORTON, 2015b), with a length ranging from 2.5 to 6 cm (MANSUR et al., 2012) (Figure 2). This form is not found among other freshwater bivalves, except among some representatives of the Dreissenidae family, native to South America. The external color of the shell varies between dark brown and greenish above and light brown below the carina, being violet internally (MORTON, 1973). The bivalve has short siphons consisting of simple openings without tentacles or papillae (MANSUR et al., 2012; MORTON, 2015a). As for the gills, it is important to highlight that the cilia form chains to select particles, which follow to the labial palps and, from there, towards the mouth. Mansur et al. (2012) described five acceptance currents on each side of the mollusk, which the authors relate to its high filtration efficiency, when compared to other bivalves, as mentioned by Sylvester et al. (2006).



Figure 2.Golden mussel *Limnoperna fortunei*. A) valve showing color pattern and triangular outline (Umbo, U; Carena, C; Topo, T); B) macrocluster collected in Lake Guaíba, Porto Alegre, Rio Grande do Sul. Photo: MCD Mansur.

According to Mansur (2012), the adult animal's foot is very small, making it difficult its locomotion when detached from the substrate. It is formed by a tissue with different cell types involved in the adhesion and locomotion mechanisms, with numerous villi, hairs and mucus throughout the external surface (FRÓES et al., 2012). Another important function of the foot is the production of byssus threads, which are secreted by a gland located on the dorsal part

¹Shell with typical shape of the genus *Modiola*, triangular with a rounded posterior region.

from the inside of the foot (MANSUR, 2012), in a region reinforced by collagen fibers, formed by several canaliculi (FRÓES et al., 2012). Ohkawa et al. (1999) purified a Dopa protein with a molecular mass of 96kDA, a precursor of byssus, whose amino acid sequences of the fragments comprise two hexapeptides and four repetitive decapeptides (Lys- (Hyp/Pro) - Thr- (Gln/Tyr) - Dopa- (Ser/Thr) - (Asp/Thr) -Glu-Tyr-Lys).

1.4 Reproduction and Life Cycle

Individuals >5 mm reach sexual maturity (DARRIGRAN et al., 1999), which occurs between 3 and 4 months (BOLTOVSKOY; CATALDO et al., 1999; DARRIGRAN et al., 1999). According to Callil et al. (2012), there is no evident sexual dimorphism, but texture and color patterns in the mantle allow the differentiation of males from females. Some authors cite rare cases of hermaphroditism among populations (DARRIGRAN et al., 1998; 1999; ULIANA et al., 2006; GIGLIO et al., 2016), which do not exceed 0.6% (BOLTOVSKOY et al., 2015a), but even in these cases the male and female gametes are developed in distinct follicles (CALLIL et al., 2012).

Gametes are released by male and female individuals into the water column, where they are fertilized externally to the animal (DAMBORENEA; PENCHASZADEH, 2009; MAN-SUR, 2012). As for egg fecundity, there are no data to date (KARATAYEV et al., 2015). According to Boltovskoy et al. (2015a), studies on the gametogenic cycle in South America demonstrate that sperm and mature eggs were recorded throughout the year, with several intermittent propagation events, resulting in relatively continuous reproduction marked by seasonal peaks in spring and late summer, with larval production for 6 to 10 months throughout the year. According to the same authors, in Hong Kong there are two annual reproductive peaks, which are limited by low temperatures, and in Japan larval production is restricted to 1-2 months in the warmest periods. According to Morton (1982), the onset of gametogenesis is probably associated with multiple factors, especially those related to water quality. Pereira (2014) found that the release of larvae in spring was related to increased conductivity, alkalinity and sulfate content. Boltovskoy et al. (2015a) speculate that in addition to temperature, other factors such as pH, salinity, dissolved oxygen, suspended solids, chlorophyll *the* and flood/drought cycles trigger the reproductive cycle.

Santos et al. (2005) described the larval development stages that make up the planktonic phase of a golden mussel population in Lake Guaíba, RS, Brazil, based on plankton samples, and identified the following stages (Figure 3): valveless planktonic stages, A) ciliated morula, B) to E) trochophores I to IV (respective lengths of 80-125 µm); valved stages, F) the "D" larva (100-130 µm), G) straight hinge veliger (140-180 µm), H) umbonate veliger (190-230 µm) and I) pediveliger (230-270 µm). According to

Mansur et al. (2012), after phase I, the veil is absorbed and the foot is developed so that the animal leaves the plankton and recruits the substrate, thus constituting the beginning of the benthic phase; in the next phase (J), the post-larva or plankton antigrade ($\approx 300 \mu\text{m}$) secretes byssus threads through the gland byssogenic of the foot, allowing adherence to the substrate, when it acquires epifaunal habit (Figure 3). In the benthic phase, *L. fortunei* will live for a period of 2 to 3 years, a slightly shorter longevity, when compared to the invasive Dreissenidae, which reach up to 5 years (KARATAYEV et al., 2015).

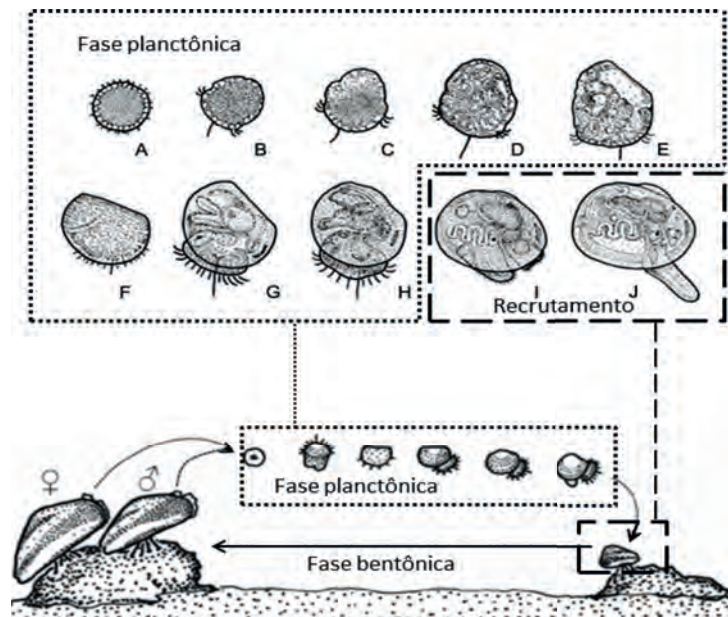


Figure 3. Life cycle of the golden mussel: A) ciliated morula, B) to E) trochophores I to IV (respective lengths of 80-125 μm); valved stages, F) the "D" larva (100-130 μm), G) straight hinge veliger (140-180 μm), H) umbonate veliger (190-230 μm), I) pediveliger (230-270 μm) and J) the postlarva or plantigrade (300 μm) benthic epifaunal. Source: adapted from Mansur et al., 2012.

1.5 Habitats

The golden mussel is a freshwater species that is not very selective in terms of habitat. aquatic species, and can live in coastal regions of lakes, lagoons, lagoons and dams, river banks and channels and streams. However, this species has a certain tolerance to brackish waters, with records in estuarine regions of Asia, where salinity does not exceed 13‰ (RICCIARDI, 1998). On the other hand, it is more restrictive regarding salinity values (<2‰), in which the mollusk has established populations (MORTON, 1976).

Colling et al. (2012) demonstrated that the golden mussel's advance into the estuarine area of Laguna dos Patos was due to climate events such as El Niño, which led to a decrease in salinity in the estuary due to the greater discharge of freshwater. After these limnification scenarios that occurred between 2002-2003 and 2009-2010, population control occurred naturally due to the increase in salinity, which caused the mortality of the species in the estuary. After the event, only populations were observed in the limnic part of the lagoon. This small window of time of predominance of limnification may have been sufficient to expand the distribution of the mollusk in tributaries and spillways, constituting the probable entry point for this invader into Laguna Mirim.

The golden mussel has colonized different aquatic environments in South America such as streams, rivers, dams, lakes, coastal lagoons, lagoons in low salinity scenarios, as previously mentioned, and river deltas (CORREA et al., 2015). It can be found in different mesohabitats and microhabitats previously occupied only by non-encrusting organisms. The only native organisms that attach themselves to hard substrates are species of Porifera, Bryozoa, bivalves Sphaeriidae of the genus *Eupera*, which attach themselves to aquatic vegetation by means of byssus threads, without forming clusters (MANSUR; VEITENHEIMER, 1975), as well as species of Dreissenidae of the genera *Mytiliopsis*, native to the Amazon Basin, which form very small clusters (ALVARENGA; RICCI, 1989) when compared to the golden mussel.

In streams and rivers, the golden mussel can occur in both mesohabitats of rapids as a backwater, attached to submerged hard substrates (Pereira, personal observation). In coastal lakes and lagoons, it can be found both on the coast and in deep zones, on hard substrates or on other organisms, which have a rigid carapace allowing attachment by byssus threads. The occurrence in deep zones is scarcer and may tend to zero, depending on the depth of the lentic body. In deltaic systems, they are found in distributary channels between islands and small inlets, called sacs, where they adhere to aquatic macrophytes (PFEIFER; PITONI, 2003). In floodplains of large rivers, they occur in bays (OLIVEIRA; BARROS, 2003). In dams, the distribution depends on the depth, flow and composition of the substrate, and may occur up to approximately 14 m deep on sand and pebble substrates and rocky bottoms (CORREA et al., 2015). In lagoons, habitat distribution varies depending on the saline wedge inlet, with a predominance of records in estuary limnification scenarios and high mortality rates in saline wedge inlet scenarios (CAPÍTOLI et al., 2008).

In the larval phase, the golden mussel occupies the water column, integrating the plankton. At this stage, they can reach a density of 30,000 ind./m³ (PEREIRA, 2014), presenting seasonal fluctuations, with the absence of larvae in colder months, as well as in warmer months, when the water level decreases. As adults, the mussels occupy substrate

preferably consolidated, but they can also colonize unconsolidated substrates. Several substrates are cited in the literature (Figure 4), such as: rocky shores and bottoms, branches of emergent and floating aquatic macrophytes (*Scirpus californicus*, *Juncus* spp., *E. crassipes*, *E. azurea*) (MANSUR et al., 2003), roots and branches of riparian vegetation (*Cephalanthus glabratus*) (MANSUR et al., 2008), mollusk shells (MANSUR et al., 1999; 2003; SANTOS et al., 2012a), crustacean carapaces (LOPES et al., 2009) and turtle shells (CARDOSO, 2014), the latter being only based on laboratory experiments. In the absence of hard substrates, it can also adhere to stabilized silt, therefore, the absence of hard substrates does not constitute an obstacle to the colonization of aquatic environments (CORREA et al., 2015).



Figure 4. Natural substrates used by *Limnoperna fortunei* in Lake Guaíba, RS, Brazil: A, settlement on the rhizome of the "junco" *Scirpus californicus*; B, mussel bed on *Scirpus californicus* in the second year of invasion; C, rushes in 2001, before the invasion; D, landscape modification, after 2 years of settlement in the rhizomes; E, cluster suffocating the native bivalve *Diplodon* sp.; F, cluster suffocating the native bivalve *Leila blainvilliana*; G, in the gastropod's umbilical cord *Pomacea canaliculata* (LAMARCK, 1822); H, clusters on branches of the "sarandi" *Cephalanthus glabratus*; I, rolled shells, modifying the landscape of the beaches in the shore (Photos: MCD Mansur). Source: Santos et al. (2012a).

Artificial substrates present in man-made environments, as well as surfaces of means of transport, are also colonized by this species, including: grids, turbines, stakes, cooling systems, concrete walls, water collection pumps, reservoir gates, piers, boats and jet skis (MAN-SUR et al., 2012; CORREA et al., 2015) (Figure 5).



Figure 5. Artificial substrates used by *Limnoperna fortunei*: 3A, clogged self-cleaning filter; 3B, detail of the lid; 3C, cylinders inside the same filter; 3D, beginning of the cleaning process of a pump collecting encrusted water; 3E, pump hoisted by crane from the roof; 3F, pump protective grid; 3G, marina pier; 3H, hull of a boat used for fishing in Lake Guaíba and Lagoa dos Patos, being transported by road to another source, posing a risk of contamination. Photos: A, B, C: Gustavo Darrigran, www.malacologia.com.ar; D, E, F: José Imada, CORSAN; G: Augusto Chagas: *Biociências*, 2004, 13(1); H: MCD Mansur: *Brazilian Magazine of Zoology* 2003, 20(1). Source: Santos et al. (2012a).

1.6 Population Dynamics

According to Karatayev et al. (2015), *L. fortunei* reaches a maximum size of 30 mm, comparable to the size of the zebra mussel. According to Nakano et al. (2015), in subtropical regions, the golden mussel reaches 20 mm in the first year of life and 30 mm in the second year. In a population from the port area of Porto Alegre, in Lake Guaíba, in Rio Grande do Sul, Mansur et al. (2012) recorded an exceptional size of 60 mm in length. The longevity of *L. fortunei* comprises a period of 2 to 3 years (KARATAYEV et al., 2015). According to the authors, the growth rate depends on the temperature while the length of periods with high temperatures.

The authors evaluated growth curves of the golden mussel in the Upper Paraná, where temperatures are above 20 °C and found that the bivalve can reach more than 35 mm in length in the first year of life, while in the Lower Paraná River (10-28 °C) it grows up to 20 mm in the first year. Relationships between the size and biomass of populations of *L. fortunei* vary between water bodies and periods of the year, depending on feeding conditions (NAKANO et al., 2015). About 93% of individuals less than 1 mm die before reaching 2 mm and 80% of mussels measuring 22 mm reach 20-23 mm (SYLVESTER et al., 2007).

1.7 Limiting Factors and Environmental Tolerance

The golden mussel has a high tolerance to water pollution, tolerates concentrations of very low calcium levels (up to 1 mg/L), which favors its dispersion in calcium-poor waters of Brazilian rivers and lakes; the zebra mussel tolerates a minimum value of 23 mg/L, which would probably not make its survival viable in most of Brazil's river basins (KARATAYEV et al., 2015). The golden mussel requires a minimum temperature of 15 °C to reproduce, while the zebra mussel requires 12 °C and the guaga mussel 5 °C, which also makes it difficult to maintain populations of these dreissenids if they are introduced into Brazilian waters. As for salinity, the golden mussel tolerates continuous values of 2‰ and discontinuous values of up to 23‰, which is why it survives low salinity scenarios in the Lagoa dos Patos estuary. As for the minimum and maximum temperatures for the survival of adult populations, the authors point to 0 and 35 °C for the golden mussel.

In the Pantanal, there is a wide variation in the limnological characteristics of the water, when compared to other water systems in South America. Oliveira (2003) and Oliveira et al. (2006, 2011, 2013) found that scenarios of dissolved oxygen depletion, low calcium content, reduced pH, low chlorophyll *the*, as well as high water velocity and high concentration of suspended solids affect the golden mussel populations, resulting in a decrease in population density, due to the synergy of these variables. The authors, in

However, they emphasize that this event probably does not prevent the persistence of populations in the Pantanal and the establishment of viable populations in upstream headwater areas.

According to Karatayev et al. (2015), the golden mussel tolerates up to 0.5mg/L of dissolved oxygen. Oliveira et al. (2010a) evaluated the mortality of golden mussels in a floodplain lake in the Pantanal, where oxygen depletion events can last for weeks during the flood phase, and found mortality of these organisms after 5 days. They also found that a population established in the same lake in 2005 was extirpated in 2006 due to hypoxia. Laboratory tests performed by the authors corroborated the field data and suggest that annual oxygen depletion may control the densities of this invader, potentially resulting in the disappearance of populations in some habitats.

Laboratory tests demonstrate the survival of the golden mussel in values extremes of pH (4-11), after 5 days of exposure (PEREIRA, 2014). This pH tolerance range is comparable to the range tolerated by the dipteran *A. aegypti*, a mosquito that carries dengue fever (CLARK, 2004). Furthermore, it also indicates that there is a risk of the mussel spreading when it passes through the digestive tract of fish that do not have a mouth apparatus adapted to crush the shells, allowing the animal to survive after being released through the cloaca, since some fish have a stomach pH between 2 and 6 (ROTTA, 2003).

1.8 Trophic Relationships

This item discusses the trophic relationships of the golden mussel in relation to its role as a suspension filter feeder, especially regarding its diet, as well as the predation of larvae, recruits and adults, by the fauna of the invaded areas.

1.8.1 Golden Mussel Feeding

The golden mussel is an active filter feeder, feeding on suspended material, such as phytoplankton, zooplankton, bacteria and suspended solids. Its filtration capacity is much higher when compared to other freshwater bivalve species such as *Corbicula fluminea* and marine species of Mytilidae and Veneridae, as well as having a higher filtration rate than some species of *Dreissena*, when at temperatures above 20 °C (SYLVESTER et al., 2009). According to this author, phytoplankton can supply 97% of the basic energy demands of a 23 mm long mussel, based on laboratory information. Through density estimates, along 5 km of the Luján River (Argentina), with different substrates, and based on the mollusk's filtration rates (200 mL mussel⁻¹.h⁻¹), they also highlight that *L. fortunei* can filter a third of the water volume of this river, causing severe impacts on the planktonic community.

The selectivity of phytoplankton species in the diet of *L. fortunei* is widely explained by a combination of cell shapes, biovolume and available phytoplankton taxa (FRAU et al., 2016). Fachini et al. (2012) evaluated the feeding selectivity of *L. fortunei* in the laboratory and found that there was a greater preference for spiny algae (genera *Desmodesmus*, *Kirchneriella*, *Monactinus*, *Pediastrum*, *Scenedesmus*, *Staurastrum* and *Tetrahedron*) and filamentous (genera *Aulacoseira*, *Mougeotia*, *Planktothrix*). Experiments carried out by Frau et al. (2016) reveal that this mollusk prefers algae with a maximum linear dimension of 20 to 100 µm, representatives of Desmidiaceae, Chlorococcales, Euglenophyceae and Chrysophyceae. The food selectivity of the golden mussel when offered food consisting of cyanobacteria was evaluated in Gazulha et al. (2012a, b), and the mollusk's food preference for toxic strains was verified, which indicates that it must present high resistance to cyanotoxins.

Another study reveals the predominance (67%) of the biomass of zooplankton organisms in the diet of golden mussels, with representatives of Protista, Rotifera, Nematoda, Cladocera, Copepoda, Ostracoda, in addition to their own larvae, accounting for a total of 156 taxa (MOLINA et al., 2015). In the experiments by Fachini et al. (2012), regarding the selectivity of zooplanktonic organisms, the authors verified the preference for rotifers (genera *Conochilus*, *Bdelloidea*, *Lecane*, *Lepadella* and *Trichocerca*; *Brachionidae*), of the zooplanktonic organisms. The authors verified an average filtration rate of 63 mL mussel-1h-1, pseudofeces production of 34 mL mussel-1h-1 and ingestion of 28 mL mussel-1h-1. Microcrustaceans can constitute an important food source for the invasive bivalve, which can ingest microcrustaceans larger than 1,100 µm, a size range larger than that presented by microcrustaceans preyed upon by *Dreissena polymorpha* in invaded environments (MOLINA et al., 2011). Rotifers are the planktonic prey most frequently preyed upon by the golden mussel, followed by cladocerans and copepod nauplii, which are positively selected for by size (MOLINA et al., 2015). The contribution of phytoplankton and zooplankton to the diet of the golden mussel may vary between ecosystems. Furthermore, laboratory studies may reveal habits that are distinct from those occurring in the ecosystem.

1.8.2 Predation of Golden Mussels

Regarding the larval stages of fish, Paolucci et al. (2010) present important data many about the species *Prochilodus lineatus* (curimbatá). The authors found that the larvae of this species fed intensively on veliger larvae of the golden mussel. In experiments comparing the diet consisting of zooplankton enriched with veligers of the mollusk and without enrichment (control), to evaluate the effects of the diets on the growth of the fish larvae, the authors found that the diet enriched with veligers of *L. fortunei* significantly improves the growth of larvae *P. lineatus*, which possibly has a preference for this food resource, which has a higher energy content when

compared to other resources, in addition to being more easily captured due to low mobility. Paolucci and Thuesen (2015) found that larvae of 18 species, including the most abundant members of Characiformes and Siluriformes, feed on golden mussel veligers in the Río de la Plata Basin.

Of 157 specimens of the fish *Leporinus obtusidens* (20-55 cm total length) collected in the Rio de la Plata, Costanera Norte, in Buenos Aires, 98 (72.1%) contained fragments of shells of *L. fortunei*, covering 14.5% of the dry weight of stomach contents and 44.4% of intestinal contents (PENCHASZADEH et al., 2000). Lopes and Vieira (2012) found the presence of golden mussels in the stomach contents of 10 species of fish in the shallow areas of the São Gonçalo Channel and Lagoa Mirim, Rio Grande do Sul: *Crenicichla punctata* (frequency of occurrence = 96.3%); *Astyanax fasciatus* (72.2%), *Rineloricaria microleptogaster* (55.6%), *Painted Pimelodus* (52.4%), *Micropogonias furnieri* (41.7%), *Geophagus brasiliensis* (42.1%), *Rhamdia* *what* (31%), *Hoplias malabaricus* (20%) and *Hypostomus commersoni* (2.4%). Isaac et al. (2014) found that golden mussels were used in the diet of 15 fish species from three subsystems of the Upper Paraná River floodplain (Brazil), which are listed below in decreasing order of importance: *Leporinus obtusidens*, *Leporinus friderici*, *Leporinus macrocephalus*, *Leporinus lacustrines*, *Geophagus* *proximus*, *Hoplias* *malabaricus*, *Hoplosternum littorale*, *Leporinus elongatus*, *Leporinus macrocephalus*, *Pimelodus maculatus*, *Potamotrygon* *f. falconers*, *Pterodoras granulosus*, *Schizodon borellii*, *Schizodon nasutus*, *Serrasalmus maculatus*, *Trachydoras paraguayensis* and *Serrasalmus marginatus*.

In the Ilha Solteira reservoir, in the Upper Paraná River, Rosa et al. (2015) verified the presence of the golden mussel in the digestive tract of 13 species of fish, highlighting *Leporinus obtusidens*, *L. friderici* and *Piaractus mesopotamicus* due to greater consumption, which presented broken shells in the stomach and intestine, evidencing the efficiency of the mouth apparatus in crushing these animals. Other species, *Pimelodus maculatus* and *Geophagus proximus*, had intact individuals, which may pose a risk to the dispersion of the invading bivalve, as it may survive passage through the digestive tract.

Leporinus obtusidens, *Rhinodoras dorbignyi* and *Brochiloricaria chauliodon* presents *L. fortunei* as part of their diet, according to observations of stomach contents carried out by García and Montalto (2006). The authors also highlight the risk of contamination by heavy metals for the human population that consumes these predatory fish of the mollusk, which has a high potential for bioaccumulation of toxic metals (SOARES et al., 2009; VILLAR et al., 1999).

In his review of the predation of golden mussels by ichthyofauna, Cataldo (2015) highlights that the list of predatory fish species *L. fortunei* is constantly increasing. About 18 species were identified by 2006, and after 7 years, 50 species made up the list. Furthermore, the author found that: > 50% of the species are among the regularly

present in commercial fisheries, with omnivorous, detritivorous and ichthyophagous guilds prevailing; economically important species have replaced a diet consisting of plants and detritus with one dominated by adult mussels; fish lacking adequate dentition to crush shells also swallow whole specimens or the siphons and exposed edges of the mantle; in some cases, 100% of the intestinal content is composed of the invasive bivalve, especially in summer; selective consumption of smaller size classes of mussels prevails; predation pressure is probably high, constituting a significant mechanism for modulating mussel populations. However, according to the authors, predation pressure is probably insufficient to eradicate this invasive species.

Cardoso (2014) evaluated the predation of the golden mussel by five species of Chelonia of the coastal plain of Rio Grande do Sul, in laboratory experiments, and found that *Phrynops hilarii* (Chelidae) and *Trachemys dorbigni* (Emydidae) consumed respectively 40% and 37% of the mussels offered in the experiments, while the other species (*Acanthochelys spixii* and *Hydromedusa tectifera*) consumed <2%. The presence of *L. fortunei* in the diet of *Trachemys dorbigni* had already been recorded by Bujes et al. (2007) in a population from the Jacuí River Delta, Rio Grande do Sul.

1.8.3 Mitochondrial Genome

The mitochondrial genome of *L. fortunei* was recently described in Uliano-Silva et al. (2016). The authors found that the mitochondrial genome of this invasive species does not present a conserved gene arrangement when compared to other Mytilidae species, which suggests a high degree of gene recombination in the mitochondria of this clade. In addition, it encodes two copies of tRNA^{Lys} and presents a putative pseudogene for the sequence of the *atp8* gene, which encodes a 27-amino acid peptide containing a stop codon in the frame. Phylogenetic analysis of the complete available mitochondrial genome of Mytilidae confirms the strong evolutionary relationship between species of *Mytilus*, while *L. fortunei* is positioned in a more ancestral branch of the family. Uliano-Silva et al. (2015a) highlight that the HSP70 gene of *L. fortunei* may be related to the success of the golden mussel invasion, since in a phylogenetic analysis of HSP70 in molluscs, Uliano-Silva et al. (2014) found that two isoforms of HSP70 in *L. fortunei* are evolutionarily related to the expansion observed in *Crassostrea gigas*. It also identified two proteins related to byssus (Mepf1 and Mepf2), in the transcription of the golden mussel. Regarding Cytochrome P450, according to Uliano-Silva et al. (2015b), *L. fortunei* has a set of CYP3A genes that are not phylogenetically related to CYPs from any other bivalve. According to the authors, these genes are related to the ability to withstand environmental stresses during the invasion process. Uliano-Silva et al. (2014) also point out that the transcriptome of this invasive species revealed eight genes involved in the receptor signaling pathway, which may be related to an adaptive immune system.

2. Invasion Process

The invasion process in continental waters comprises five stages, some of which are related to obstacles that the invasive species must overcome: survival of transport, release into the aquatic environment, establishment of the population, dispersal throughout the invaded area and impacts of the invasion (DARRIGRAN; DAMBORENEA, 2009). The golden mussel was introduced into South America unintentionally via ballast water. The species survived transport and established populations initially in Balneario Bagliradi, in the estuary of the La Plata River, in Argentina, through which it dispersed throughout the La Plata Basin, among others, generating environmental and economic impacts. The next item summarizes the invasion process based on the invasion chronology, introduction routes, dispersal vectors and potential future invasion and dispersal scenarios in new areas in South America and other continents.

2.1 Native Geographic Distribution

The native distribution of *L. fortunei* was probably restricted to the Pearly River Basin, in China (XU, 2015; MORTON, 1973).

2.2 Invaded Areas and Invasion Chronology

The chronology of invaded areas, widely described and discussed by several authors, (DARRIGRAN; MANSUR, 2009; XU, 2015; ITO, 2015; OLIVEIRA et al., 2015), supports the understanding of the invasion process and the simulation of new invasion and dispersion scenarios.

2.2.1 Asia

In China, from the Pearly River Basin, the golden mussel was transferred by ships during the 1960s and 1970s to river estuaries in the Fujiang and Zhejiang regions, as well as the Yangtze River (XU, 2015). The same author comments that after 1980 the species expanded its geographic distribution to the Huaihe, Yellow and Haihe River basins; to Tianjin, a city on the Bohai Sea in northern China; and areas around Beijing. Before 1960, it was probably introduced by humans to Cambodia, Vietnam, Laos, Thailand, and between 1960 and 1980, to South Korea (XU, 2015; MORTON; DINESEN, 2010). In Japan, Ito (2015) reports the chronology of the invasion, which began in 1990, in the hydrographic system of the Kiso-Nagara-Ibi rivers; in 1992, new records were reported for Lake Biwa and the Yodo River; the Yahagi River and Tenryu Rivers presented records of the invader in 2004; later, records followed in 2005, for Lake Kasumigaura and the Kabura-gawa irrigation canal; in 2007, the Uren and Tone Rivers were invaded, and in 2008 the Edo River.

2.2.2 South America

The chronology of the invasion in South America is described in detail in Darrigran and Mansur (2009), for the period from 1998 to 2004, and Oliveira et al. (2015) until 2015 (Figure 6). In summary, the species was initially introduced in South America in Argentina in the early 1990s (DARRIGRAN; PASTORINO, 1995), with the first record being observed in Balneário Blagliardi, in the estuary of the La Plata River. From there, it dispersed along the Argentine side of the estuary and, later, along the Uruguayan side, colonizing an extensive area of the La Plata River estuary. In mid-1998-1999, the first records were verified in Brazil, in Rio Grande do Sul, in Lake Guaíba (MANSUR et al., 1999), in Lagoa Mirim (BURNS et al., 2006) and in the Jacuí Delta (MANSUR et al., 2003). In this same period, the invasive bivalve dispersed through Uruguayan river basins, tributaries of the La Plata River estuary. Records from 1997 for the Paraguay River, in Asunción (Paraguay), and from 1998 for Corumbá, in Mato Grosso do Sul (Brazil). This shows the broad spectrum of geographic distribution already in the first years of invasion (OLIVEIRA et al., 2015). *L. fortunei* advanced 240 km/year upstream in the La Plata Basin (DARRIGRAN; EZCURRA DE DRAGO, 2000). This dispersion advance occurs mainly through the transport of individuals attached to the hulls of vessels transported by water or land. In 2001, dispersion advanced with records at the Itaipu Hydroelectric Power Plant, Paraná River Basin, in Brazil, and at the Uruguay River, in Uruguay (DARRIGRAN; MANSUR, 2009). According to Darrigran and Ezcurra de Drago (2000), considering the Itaipu dam to be an insurmountable barrier for the golden mussel to invade the reservoir, two hypotheses are presented: 1) accidental introduction by birds, mammals or humans; and 2) by means of boats, which were purchased in Porto Alegre and transported to the reservoir. One year after detection in Itaipu, 8,000 individuals were verified at the dam (ZANELLA; MARENDA, 2002). In the large Lagoa dos Patos basin, between 2005 and 2012, the dispersion reached tributaries of Lago Guaíba, being recorded in the Upper Jacuí River, Rio dos Sinos (MANSUR; PEREIRA, 2006; HÜBEL et al., 2009), Gravataí River, Taquari River and Caí River (TERRA et al., 2007). Between 2009 and 2013, the first records were made in the Tramandaí River Basin, including the Peixoto, Quadros and Itapeva lagoons. Between 2001 and 2005, the greatest dispersion of the mussel occurred in the Upper Paraná River, reaching several reservoirs along this river and the Tietê River. In 2011-2012, it was detected in the Furnas reservoir, in Rio Grande, in Minas Gerais (Biologist Paulo Formagio, personal communication) and in the Upper Rio Uruguai, at the Barra Grande HPP, in Rio Pelotas (AGUDO-PADRÓN; PORTO FILHO, 2013).



Figure 6. Chronology of the invasion and dispersal of *Limnoperna fortunei* in South America, prior to the record in the São Francisco River. Source: Oliveira et al. (2015).

In 2014, it advanced into Mato Grosso to the Taimã Ecological Station (OLIVEIRA et al., 2015). The most recent record was in the northeast of the Continent, at the Sobradinho HPP, on the São Francisco River (BA) (BARBOSA et al., 2016).

Considering the major hydrographic regions of South America (Figure 7), the golden mussel has spread along the Southeast Atlantic Coast (Argentina), Grandes Salinas and Mar Chiquitita (Argentina), the East Atlantic Coast (from Uruguay to Brazil, in the state of Paraná), throughout practically the entire La Plata Basin (Brazil, Argentina, Uruguay and Paraguay) and, finally, it is found in the São Francisco River, covering part of the states of Bahia and Pernambuco (BARBOSA et al., 2016). It is absent from the other hydrographic regions and countries of this Continent, associated with the Pacific and North Atlantic coasts. Countries on the Pacific coast have less navigation activity than countries on the Atlantic coast, which, together with the fast flow of the rivers, make it less likely to sustain populations of *L. fortunei* in their hydrographic systems (BOLTOVSKOY, 2015).



Figure 7. Distribution of *Limnoperna fortunei* in South America, based on information from the www.cbeih.org/ database (CBEIH, 2017).

The invasion process of the golden mussel in Brazilian waters occurred due to several introduction events, unnatural vectors that transported propagules from various locations (SILVA, 2012). Analyzing 24 populations of *L. fortunei* (10 from Asia and 14 from South America), based on the mitochondrial cytochrome c oxidase subunit I (COI) gene and eight polymorphic microsatellite markers, Ghabooli et al. (2013) investigated the population genetic structure in invaded areas and native distribution areas. The authors concluded that introduced populations in Asia exhibit greater diversity than in South America, suggesting greater introduction effort for invasive populations from Asia. Furthermore, they found that there is a pronounced geographic structuring in the invaded regions and a fine-scale genetic structuring in both continents, suggesting multiple introductions of distinct propagules or strong post-introduction selection, as well as demographic stochasticity. They also conclude that the greater genetic diversity in Asia may be associated with greater propagule transport activities from source and donor regions to invaded areas.

2.2.2.1 Reservoirs

Through bibliographic compilation and the database of the Bioengineering Center, According to the Hydroelectric Invasive Species Registry (CBEIH) (base.cbeih.org), to date, records of the golden mussel have been found in 50 reservoirs (Table 2, Figure 8), 7 of which are distributed along the Upper Jacuí River (RS), 1 in the Canoas River (SC), 1 in the Pelotas River (SC/RS), 3 in the Uruguai River (SC/RS), 5 in the Iguaçu River (PR), 2 in the Jordão River (PR), 1 in the Tibagi River (PR), 7 in the Paranapanema River (SP/PR), 6 in the Tietê River (SP), 4 in Paraná (PR, SP/MS, MS/SP and SP), 9 in Grande (seven in SP/MG and one in MG), 2 in the Paranaíba River (MG), 1 in the Claro River (GO) and 1 in the São Francisco River (BA). Invasion is a dynamic and continuous process, depending on the survival of the invasive species during transport (vectors) and the breaking of ecological barriers, and the establishment of prevention, control or eradication measures is extremely related to these factors (DARRIGRAN et al., 2012). Many hydroelectric plants are probably in the process of colonization at this time, however, with no records or with records not yet disclosed by their managers. Transparency and rapid communication between managers, researchers and all water users of a hydrographic system would facilitate the implementation of assertive measures to prevent introduction, dispersion, control and monitoring, with a better return on investment in a shorter period of time (DARRIGRAN; DAMBORENEA, 2009).

Table 2. Brazilian hydroelectric plants with records of golden mussel occurrence. The location coordinates listed refer to the axis of the dams, approximately.

Hydroelectric Power Plants	Location		UF	River	Bowl	Company	Source
	Latitude	Longitude					
Ernestina	- 28.551731	- 52.536922	RS	Jacui	Ducks Lagoon	CEEE	Oliveira et al., 2015
Itauba	- 29.260229	- 53.235359	RS	Jacui	Ducks Lagoon	CEEE	Santos et al.(2012a)
Royal Step	- 29.016177	- 53.182810	RS	Jacui	Ducks Lagoon	CEEE	Santos et al.(2012a)
Leonel de Moura Brizola	- 29.073024	- 53.208440	RS	Jacui	Ducks Lagoon	CEEE	Santos et al.(2012a)
Capingui 1	- 28.346209	- 52.220115	RS	Jacui	Ducks Lagoon	CEEE	Oliveira et al. (2015)
July 14th HPP	- 29.064257	- 51.674431	RS	Tapirs	Ducks Lagoon	CERAN	CBEIH (2017)
Mrs. Francisca	- 29.449123	- 53.285141	RS	Jacui	Ducks Lagoon	Dona Consortium Francisca	Oliveira et al., 2015
New Fields	- 27.601174	- 51.316443	SC	Canoes	Uruguay River	ENERCAN	CBEIH (2017)
Big Bar	- 27.776912	- 51.187888	RS/SC	Balls	Uruguay River	BAESA	CBEIH (2017)
Hatchet	- 27.527287	- 51.789773	RS/SC	Uruguay	Uruguay River	TRACTEBEL Energy	CBEIH (2017)
Ita	- 27.276223	- 52.381653	RS/SC	Uruguay	Uruguay River	TRACTEBEL Energy	CBEIH (2017)
Mouth of Chapeco	- 27.141182	- 53.039210	RS/SC	Uruguay	Uruguay River	Mouth of Chapecó Energy gia SA	CBEIH (2017)
Governor Bento Munhoz da Rocha (Mouth of the Sand)	- 26.082847	- 51.649503	PR	Iguazu	Paraná River	COPEL	CBEIH (2017)
Governor Ney Aminthas from Bar	- 25.782849	- 52.132833	PR	Iguazu	Paraná River	COPEL	CBEIH (2017)

(Continued)

(Continuation)

Hydroelectric Power Plants	Location		UF	River	Bowl	Company	Source
	Latitude	Longitude					
Gov. Joz Richa	- 25.549522	- 53.499491	PR	Iguazu	Paraná River	COPEL	CBEIH (2017)
Santa Clara	- 25.647294	- 51.953113	PR	Jordan	Paraná River	Elejor	CBEIH (2017)
Bottom	- 25.708127	- 51.997557	PR	Jordan	Paraná River	Elejor	CBEIH (2017)
Osorio Jump	- 25.532853	- 53.032828	PR	Iguazu	Paraná River	TRACTEBEL Energy	CBEIH (2017)
Santiago Falls	- 25.649519	- 52.616164	PR	Iguazu	Paraná River	TRACTEBEL Energy	CBEIH (2017)
Gov. Jayme Canet Junior (Mau)	- 24.061738	- 50.705908	PR	Tibagi	Paraná River	TRACTEBEL Energy	CBEIH (2017)
Capybara	- 22.657478	- 51.359296	PR/SP	Paranapanema	Paraná River	Duke Energy (CTG)	CBEIH (2017)
Lucas Nogueira Garcez (Big Jump)	- 22.935632	- 50.249248		Paranapanema	Paraná River	Duke Energy (CTG)	CBEIH (2017)
Canoes I	- 22.940911	- 50.516468	PR/SP	Paranapanema	Paraná River	Duke Energy (CTG)	CBEIH (2017)
Canoes II	- 22.935632	- 50.249248	PR/SP	Paranapanema	Paraná River	Duke Energy (CTG)	CBEIH (2017)
Taquaruçu (School Polytechnic)	- 22.541472	- 51.999514	PR/SP	Paranapanema	Paraná River	Duke Energy (CTG)	CBEIH (2017)
Chavantes	- 23.128130	- 49.730918	PR/SP	Paranapanema	Paraná River	Duke Energy (CTG)	CBEIH (2017)
Rosanna	- 22.599531	- 52.868951	PR/SP	Paranapanema	Paraná River	Duke Energy (CTG)	Oliveira et al. (2015)
Beautiful Bar	- 22.518962	- 48.533985	SP	Tietê	Paraná River	AES Tietê	CBEIH (2017)
Bariri	- 22.152853	- 48.752041	SP	Tietê	Paraná River	AES Tietê	CBEIH (2017)

(Continued)

(Continuation)

Hydroelectric Power Plants	Location		UF	River	Bowl	Company	Source
	Latitude	Longitude					
Rui Barbosa (New Avanhandava)	- 21.118418	- 50.200643	SP	Tietê	Paraná River	AES Tietê	CBEIH (2017)
Three Brothers	- 20.668173	- 51.299506	SP	Tietê	Paraná River	CESP	CESP (2017)
Ibitinga	- 21.758690	- 48.990096	SP	Tietê	Paraná River	AES Tietê	CBEIH (2017) Pereira et al. (2012a)
Promise	- 21.306808	- 49.757681	SP	Tietê	Paraná River	AES Tietê	CBEIH (2017)
Itaipu	- 25.426470	- 54.592539	PR	Paraná	Paraná River	Itaipu Binational	CBEIH (2017)
Engineer Sergio Motta (Port Spring)	- 22.477503	- 52.955613	SP/MS	Paraná	Paraná River	CESP	CBEIH (2017)
Engineer Souza Days (Jupia)	- 20.775367	- 51.626190	MS/SP	Paraná	Paraná River	CESP	CBEIH (2017)
Single Island	- 20.381757	- 51.363137	SP	Paraná	Paraná River	CESP	CBEIH (2017)
Volta Grande	- 20.028418	- 48.220384	SP/MG	Big	Paraná River	CEMIG	CBEIH (2017)
Furnas	- 20.664243	- 46.319843	MG	Big	Paraná River	FURNAS	CBEIH (2017)
Jaguar	- 20.022582	- 47.434002	SP/MG	Big	Paraná River	CEMIG	CBEIH (2017)
Wasp	20.300641	- 49.196210	SP/MG	Big	Paraná River	FURNAS	CBEIH (2017)
Marshal Mascaraes' reindeer (Fish)	- 20.287302	- 47.065671	SP/MG	Big	Paraná River	FURNAS	CBEIH (2017)

(Continued)

(Continuation)

Hydroelectric Power Plants	Location		UF	River	Bowl	Company	Source
	Latitude	Longitude					
Port Colombia	- 20.123696	- 48.571492	SP/MG	Big	Paraná River	FURNAS	CBEIH (2017)
Red Water	- 19.850646	- 50.345091	MG	Big	Paraná River	AES Tietê	CBEIH (2017)
Igarapava	- 19.982861	- 47.749555	SP/MG	Big	Paraná River	Igarapava Consortium	CBEIH (2017)
Capsize	- 18.451466	- 47.985496	MG	Paranaiba	Paraná River	CEMIG	CBEIH (2017)
Saint Simon	- 19.017595	- 50.498703	MG	Paranaiba	Paraná River	CEMIG	CBEIH (2017)
Mouth of the Claro River	- 19.117317	- 50.645090	GO	Of course	Paraná River	Foz do Rio Claro Power Generation	FOZRIOCLARO (2017)
Little house	- 9.430960	- 40.827390	BA	San Francisco	Sao River Francisco	CHESF	CBEIH (2017)
							(Conclusion)



Figure 8. State and federal conservation units along watercourses colonized by the golden mussel (according to data presented in Table 3). Authors of the map: Thiago Bazan and Daniel Pereira (Table 3).

2.2.2.2 Conservation Units

Through bibliographic compilation, information provided by ICMBio and the database of the Center for Bioengineering of Invasive Species of Hydroelectric Power Plants (CBEIH) (base.cbeih.org), to date, records of the golden mussel have been found in 27 conservation units (Table 3, Figure 9) located along rivers, deltas and coastal lagoon systems, 9 of which are located in Rio Grande do Sul, 2 in Santa Catarina, 6 in Paraná, 4 in the state of São Paulo, 3 in Mato Grosso, 2 in Mato Grosso do Sul and 1 covering areas of Paraná, Mato Grosso do Sul and São Paulo.

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Table 3. State and federal conservation units next to watercourses colonized by the golden mussel. The location refers to a point closest to the colonized watercourses.

Unit	UF	Location		Bowl	Source
Delta State Environmental Protection Area from Jacui	RS	- 29.955440	- 51.254555	Lagoon of Ducks	SEMA/RS (2017)
State Park of Itapeva	RS	- 29.354507	- 49.757450	Tramandaí River	Oliveira et al., 2015
State Park of Itapuã	RS	- 30.368333	- 50.997778	Lagoon of Ducks	SEMA/RS (2017)
State Park Camaqua	RS	- 31.264041	- 51.779358	Lagoon of Ducks	Capítoli et al. 2008
State Park Spinel	RS	- 30.195167	- 57.524629	Uruguay River	CBEIH (2017)
State Park Cloudy	RS	- 27.149482	- 53.855444	Uruguay River	CBEIH (2017)
Wednesday State Park Cologne	RS	- 29.455858	- 53.267324	Lagoon of Ducks	CBEIH (2017)
Biological Reserve of Big Bush	RS	- 32.166651	- 52.707098	Mirim Lagoon	SEMA/RS (2017)
Ecological Station of Taim	RS	- 32.492054	- 52.585905	Mirim Lagoon	Guimaraes (2017)
Fritz State Park Plaumann	SC	- 27.296577	- 52.118910	Uruguay River	CBEIH (2017)
Rio State Park Canoas	SC	- 27.591238	- 51.181823	Canoas River	CBEIH (2017)
Ecological Station of Bull River	PR	- 25.938504	- 52.034655	Iguazu River	CBEIH (2017)
Rio State Park Guarani	PR	- 25.456429	- 53.128704	Paraná River	CBEIH (2017)
Protection Area Environmental Protection Guaraquetaba	PR	- 25.259180	- 48.435134	Coastal Rivers	Guimaraes (2017)

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Unit	UF	Location		Bowl	Source
Ecological Station of Guaraquetaba	PR	- 25.309880	- 48.317371	Coastal Rivers	Guimaraes (2017)
National Park Iguazu	PR	- 25.534463	- 53.806854	Iguazu River	Guimaraes (2017)
Island National Park Big	PR	- 23.700068	- 53.987517	Paraná River	Guimaraes (2017)
State Park Water hyacinth	SP	- 21.203274	- 51.520632	Paraná River	CBEIH (2017)
Protection Area Environmental Cananeia-Iguape-Peruíbe	SP	- 24.619884	- 47.394114	Riverside of Iguape	Guimaraes (2017)
State Park Devil's Hill	SP	- 22.588754	- 52.359966	River Paranapanema	CBEIH (2017)
Rio State Park of the Fish	SP	- 21.601254	- 51.736146	Paraná River	CBEIH (2017)
Guirá State Park	MT	- 17.321266	- 57.854662	Paraguay River	CBEIH (2017)
Ecological Station of Taiamã	MT	- 16.864229	- 57.508861	Paraguay River	Guimaraes (2017)
National Park-end of the Pantanal Mato Grosso	MT	- 17.682684	- 57.421585	Paraguay River	Guimaraes (2017)
State Park of the floodplains of the river Ivinhema	MS	- 22.921920	- 53.654316	Paraná River	CBEIH (2017)
Panta-State Park end of the Rio Negro	MS	- 19.634419	- 56.742791	Paraguay River	CBEIH (2017)
Protection Area Environmental Protection of Islands and Floodplains of the Paraná River	PR/ MS/ SP	- 23.260541	- 53.752233	Paraná River	Guimaraes (2017)

(Conclusion)



Figure 8. Brazilian hydroelectric plants with records of golden mussel occurrence. The coordinates listed refer to the axis of the dams, approximately (according to data presented in Table 2). Authors of the map: Thiago Bazan and Daniel Pereira (Table 2).

2.3 Introduction Routes

The introduction of the species into the South American continent occurred via inter-American navigation, continental, through the ballast water of ships (DARRIGRAN; MANSUR, 2009). Within the continent, in turn, the dispersion occurred due to the wide range of vectors related to human activities (OLIVEIRA et al., 2015). The introduction in Japan was related to aquaculture (ITO, 2015).

2.4 Scattering Vectors

According to Carlton and Ruiz (2004), vectors are means by which a species gains access to a new habitat, distinct from that existing in its original range and/or its current distribution. Numerous vectors associated with human activities and natural vectors have been related to the dispersal of the golden mussel in important works (DARRIGRAN; DAMBORENEA,

2009; SANTOS et al., 2012a; FERNANDES et al., 2012), but little is known about the effective contribution of each of them.

A pioneering study was carried out by Belz (2009) for the prediction and analysis of risk of Dispersal of the golden mussel in the Iguazu River Basin, in the state of Paraná, based on the calculation of probabilities, hazards and risks of the main vectors of this basin: river transport, sand transport, transport of sport fishing boats and transport of live fish. Regarding the transport of sand from the Paraná River (contaminated area), the author found that 93.7% of the sand was destined for civil construction, without relevant risks, while the remaining 6.3% was destined for the construction of artificial beaches in freshwater reservoirs in the interior of Paraná. Of the beaches inspected, four showed traces of intact shells, probably from recently dead individuals. Regarding the transportation of sport fishing, the analysis of 34 boats, through the collection of 110 liters of water from the fishponds and 24.5 liters from the bottom of the boats, followed by inspection of the hulls and anchors, revealed: an average of 4 larvae/boat in the fishponds, 1.5 larvae/boat in the water at the bottom, 6 adult mussels/boat. Of the fishermen interviewed, 50% acknowledged that they were possibly responsible for the dispersion of the golden mussel. Regarding the transportation of live fish, the stomachs of *Pterodoras granulosus* (Valenciennes, 1840) (Doradidae), *Satanoperca papaterra* (Heckel, 1840) (Cichlidae), *Pomatotrygon motoro* (Matterer, 1841) (Potamotrygonidae), *Iheringichthys labrosus* (Lutken, 1874) (Pimelodidae) and *Megalancistrus aculeatus* (Perugia, 1891) (Loricaridae). After removing the mussels from the contents, washing and storing them in water under aeration, the presence of live adult mussels was found in the digestive tract of *P. granulosus*, which presented a diet composed of up to 100% of these bivalves. Samples from the nurseries revealed densities between 0 and 16 ind.m⁻³. The results of the calculations of probabilities, risks and dangers of each dispersion vector demonstrated that the transport of live fish was the vector with the greatest potential for dispersing the golden mussel in that basin.

The risk of each dispersal vector in a river basin is related to the economic activities, water use, social arrangements, practices and local habits, which may differ in each hydrographic system, resulting in different invasion routes or corridors (DAR-RIGRAN et al., 2012). Therefore, studies such as the one carried out by Belz (2009), contemplating a greater diversity of artificial and natural vectors, should be carried out to assess the risks of dispersion in non-invaded basins in Brazilian territory, aiming to measure the probabilities, risks and dangers of each vector for the geographic expansion of the golden mussel. Especially to assess the risk of invasion of the sub-basins that make up the large hydrographic region of the Amazon River, as well as the basins of the Tocantins and Araguaia Rivers, which present different aspects regarding water use such as navigability, fishing, aquaculture, energy generation, agriculture, connectivity, natural vectors, among others. The study of vectors is essential for establishing preventive measures for the dispersion of invasive species in hydrographic basins.

Table 4 presents a summary of the dispersion vectors, which should be considered in preventive actions against the spread of the golden mussel in river basins without records of the species. A risk analysis, based on primary data, can assess the relevance of these vectors.

Table 4. Main vectors responsible for the dispersion of golden mussel propagules in the different stages of development of the invasive organism, which present a risk of introduction and dispersion of the golden mussel in uncontaminated basins in Brazil, especially the Amazon River Basin and the Tocantins-Araguaia River Basin. Source: Darrigran and Mansur (2009), Belz (2009).

Scatter Vectors	Propagules	Theoretical Potential Risk	Prevention Actions
Vectors associated with human activities			
Ship ballast water.	Larvae	There is a flow of large vessels to Manaus.	Deballasting on the high seas, according to Norman 20, including for cabotage vessels, originating from freshwater ports, which must debalt the exchange at sea before debalt-loading in another freshwater port (FERNANDES; LEAL-NETO, 2009).
Water from reservoirs of boats.	Larvae	Belz (2009) found a high risk of contamination, through this vector, in the Iguaçú River Basin.	Do not transport water from contaminated environments to decontaminated environments. Carry out disinfection of vessels. Carry out inspections on vessels.
Water from ponds boats.	Larvae		
Water accumulated in the bottom of boats.	Larvae		
Water for transporting fry and fish (bait).	Larvae	Suframa (2003) presented an economic feasibility study that demonstrates the potential for the development of fish farming in the states of Amazonas, Roraima, Acre and Rondônia. The growth of fish farming in net cages has grown in the Amazon, posing risks of contamination of water sources, through the release of fry grown in contaminated water.	Do not use contaminated water to transport fry. Monitor the cultivation of fry. Carry out inspections of the transport of fry via terrestrial and aquatic.

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Scatter Vectors	Propagules	Theoretical Potential Risk	Prevention Actions
Sampling instruments- scientific attire	Larvae	Niskin bottles, Van Dorn bottles, plankton nets and other water collection devices can contaminate near new areas.	Clean and disinfect collection instruments before of each sampling.
	Recruits and Adults	Nets for sampling ichthyofauna; macroinvertebrate collection nets, dredges, sieves and pots.	
Boat hulls	Recruits and Adults	Vectors widely documented for other basins (DARRIGRAN; MANSUR, 2009); Santos et al. (2012a) confer risks in other basins not infested.	Carry out anti-fouling coating and periodic cleaning. tip of the boat hulls, for removal mussel mechanics and subsequent disinfection.
Powerboating	Adults and larvae	These equipments are usually transported via land, from one basin to another;	Perform inspection of this equipment when trans- ported by land, from one basin to another.
Floating platforms and buoys	Adults and larvae	Vectors widely documented for other basins (DARRIGRAN; MANSUR, 2009); Santos et al. (2012a) confer risks in other basins not infested.	Avoid moving buoys and floating platforms along the watercourse. If necessary, remove the equipment from the watercourse for mechanical removal, disinfection, and emptying of compartments containing accumulated water. Inspect this equipment when transported via landfill. restrict from one basin to another.

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Scatter Vectors	Propagules	Theoretical Potential Risk	Prevention Actions
Net tanks	Adults	Contaminated net cages thrown into uncontaminated areas will contribute to the spread of the invader. Experimental units were installed by the Institute for Sustainable Agricultural and Forestry Development of Amazonas (IDAM), with the aim of aim of intensifying this activity in the Basin.	Perform periodic cleaning after fishing, via mechanical removal, high-pressure washing and exposure to air. The procedures must be carried out outside the water so that new contamination does not occur and so that dead mussels do not cause oxygen depletion and an increase in organic matter (OLIVEIRA et al., 2015). Perform inspection of this equipment when transported by land from one basin to another. other.
Nets and other equipment fishing grounds.	Adults and larvae	Fishing is a very important economic activity. present in the Amazon basins and Tocantins-Araguaia.	Exposing the equipment to the air, under the scorching sun and disinfection.
Transposition of basins.	Adults and larvae	Implementation of transposition project Tocantins River-São Francisco River (MOLION, 2003; ALVES, 2005) would consist of establishing a new invasion route, in which several dispersion vectors mentioned in this table will contribute to the geographic expansion of the species up to the Rio Basin Amazon.	Do not carry out basin transposition between contaminated and non-contaminated watercourses.

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Scatter Vectors	Propagules	Theoretical Potential Risk	Prevention Actions
Transportation of sand extracted from contaminated river systems for the construction of artificial beaches in non-polluted areas contaminated.	Adults	Artificial beaches are recommended for the management of turtles in the Amazon (BERNHARD, 2016.).	Do not use sand from contaminated areas. Carry out inspection of the material transported via terrestrial and aquatic.
Natural Vectors			
Aquatic macrophytes.	Adults	Contaminated floating macrophytes can be transported along with vessels or against the current by means of the winds.	Do not transport aquatic macrophytes from areas contaminated to uncontaminated areas
Migratory fish.	Adults	Malacophagous fish are potential vectors of dispersion (FERNANDES et al., 2012).	There is no way to prevent the use of the golden mussel as a food resource by fauna, nor its dispersion by natural vectors, but monitoring the contribution of these vectors can measure their importance. Species of the genus <i>Podocnemis</i> with wide distribution in the Amazon and Tocantins River Basins, as well as in floodplain forests, they are potential vectors who lack studies.
Turtle hooves.	Adults	Cardoso (2014) evaluated the potential of turtles in aquatic environments in southern Brazil and found that some species are potential vectors of dispersion of the mussel. golden	
Connectivity between basins, by means of flood.	Adults and larvae	The Amazon sub-basins are highly connected during flood pulses (JUNK; ROBERTSON, 1997).	Passive dispersion due to the high degree of connectivity of this hydrographic system will be inevitable.

(Conclusion)

2.5 Potential Future Invasion and Dispersal Scenarios

Oliveira et al. (2010b) made predictions of potential distribution of *L. fortunei* in Brazilian hydrographic systems, based on limiting factors related to shell calcification, which consists of important protection for the mollusk, among other limnological and climatic variables of ecological niche using algorithms *GarpandMaxent*. The study concludes that due to the greater tolerance of the golden mussel to factors that limit the distribution of other bivalves, as well as its great capacity for calcification of shells in calcium-poor waters, the golden mussel may become widely distributed throughout Brazil. Both models *GarpandMaxent* point to the high probability of potential occurrence of *L. fortunei* in the Solimões, Tapajós, Xingu, Madeira, Amazonas, Tocantins, Araguaia, São Francisco rivers, as well as other coastal rivers (Figure 10, Figure 11 and Figure 12).

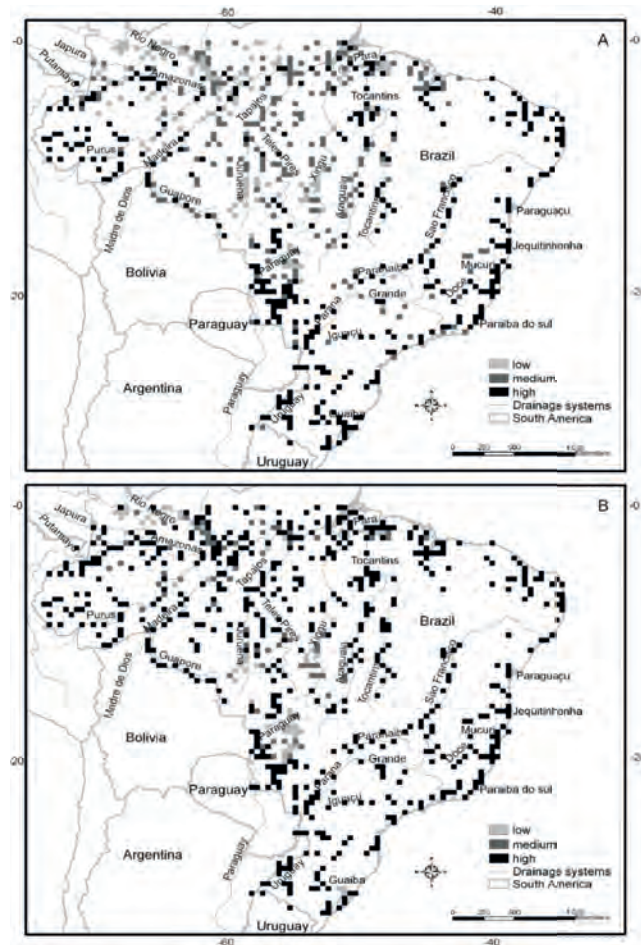


Figure 10. Potential distribution of *L. fortunei* in Brazilian river systems based on lower thresholds of calcium (A) and SI calcite (B) concentrations. We consider the low risk (pale gray) of occurrence of *L. fortunei* in rivers where the average calcium concentration was between 0.0 and 1.0 mg l⁻¹ and SI calcite was <-4.0, medium risk (dark gray) between calcium concentrations of 1.0 to 3.0 mg l⁻¹ and SI calcite between -4.0 and -3.0, and high risk (black), in which the calcium concentration was >3.0 mg l⁻¹ and SI calcite was >-3.0. Source: Oliveira et al. (2010b).

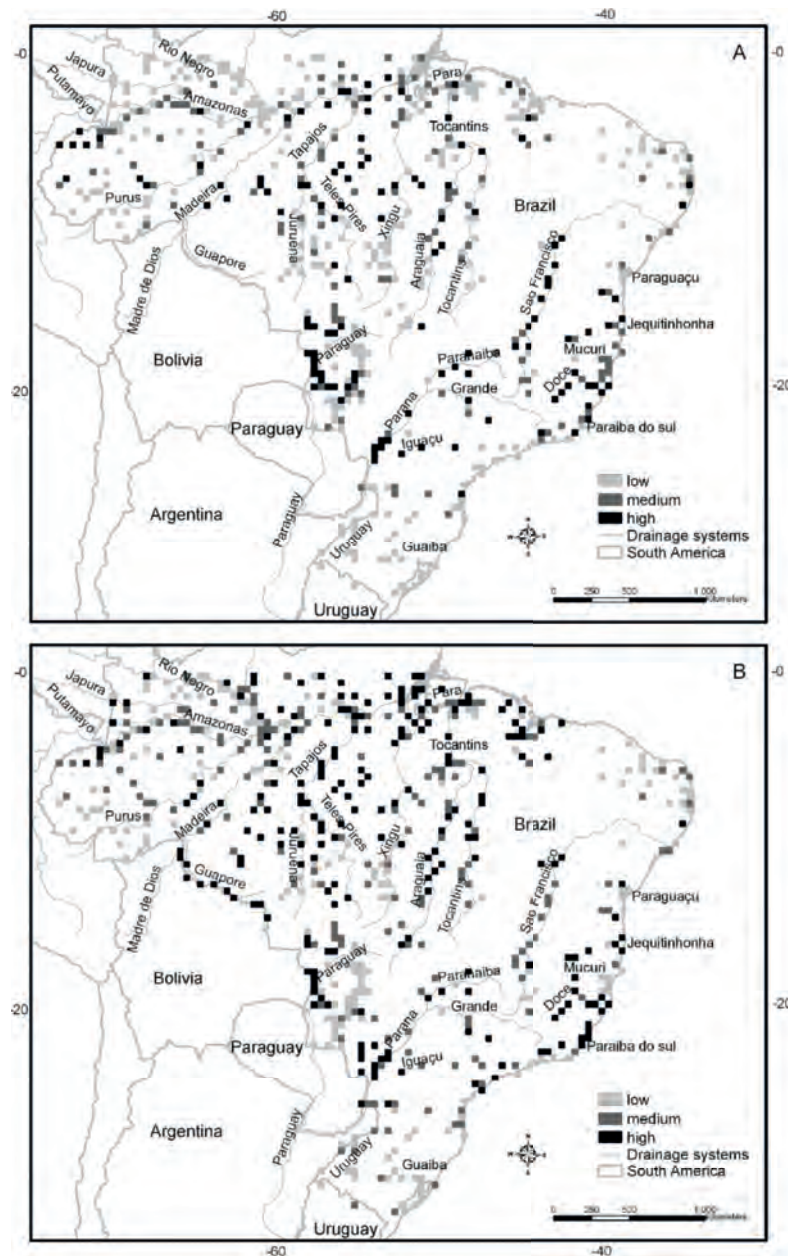


Figure 11. Predicted potential distribution of *L. fortunei* in Brazilian hydrographic systems based on the model *Garpcalibrated* for distributions observed in the Pantanal: (A) variable: Sicalcite, AUC = 0.82, Precision = 94%, omission error = 5.8% (B) variables: Calcium concentration, conductance, pH, water temperature, dissolved oxygen, AUC = 0.92, Precision = 97%, omission error = 2.9%. We consider the probability of establishing *L. fortunei* as low (pale gray) when less than 5% of the models predicted the occurrence, medium (dark gray) when 5 to 50% of the models predicted the occurrence and high probability (black) when more than 50% of the models predicted the occurrence. Source: Oliveira et al. (2010b).

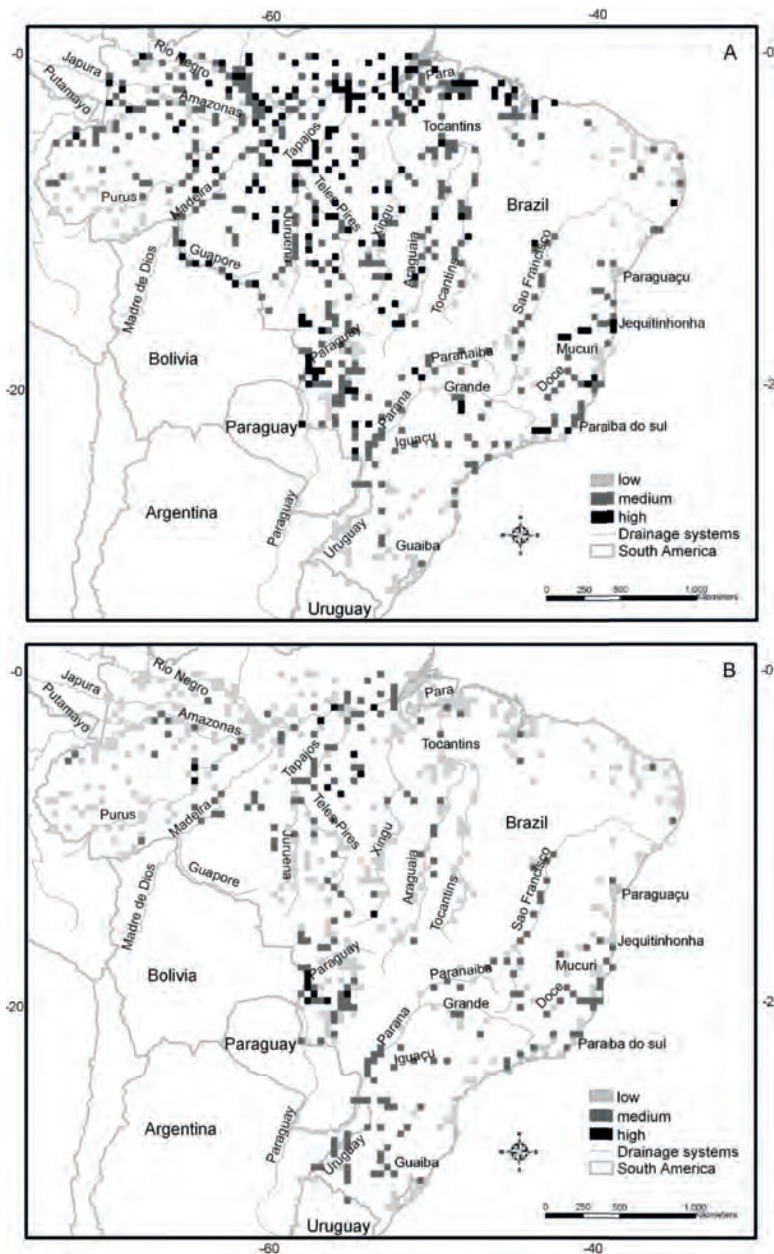


Figure 12. Predicted potential geographic distribution of *L. fortunei* in Brazilian hydrographic systems based on the model *Maxent*, calibrated based on records in the Pantanal: (A) variable: S1calcite, AUC = 0.82, threshold value = 3.0 (B) variables: calcium concentration, Conductance, pH, water temperature and dissolved oxygen, AUC = 0.96, threshold value = 5.5. *Maxent* produces a continuous forecast with values ranging from 0 to 100, in regions with better predicted conditions: light gray (between 0.0 and threshold value, low risk); dark gray (between threshold value and 50, medium risk); black (50-100, high risk). Source: Oliveira et al. (2010b).

The same type of prediction using the variables mentioned above and values of water temperature, dissolved oxygen, pH and total suspended solids was carried out by Oliveira et al. (2010c) to assess the potential distribution of the golden mussel in the Upper Paraguay River. The study concludes that the Cuiabá and Miranda rivers exhibit a high risk of invasion, while the middle part of the basin, where there is a low concentration of calcium and carbonate minerals, the risk is lower. Also using the algorithm *Maxent*, Campos et al. (2016) made predictions of the distribution of golden mussels in the Upper Paraná River, based on limnological variables and shear force. Significant spatial differences demonstrate the importance of hydrodynamic aspects in the spatial distribution of *L. fortunei*.

Campos et al. (2014) performed predictive modeling of the distribution of me-golden shilling using the algorithms *Mahalanobis Distance, Domain, Garpand Maxent*, based on data on native and invaded occurrences from Asia (71 points) and South America (248 points), compiled from the literature and from Bioclim environmental layers related to air temperature and precipitation. The scenarios obtained through this modeling show a high probability of potential occurrence of *L. fortunei* in aquatic environments of Central America, North America, Europe, Africa and Oceania, as well as expansion of the areas of occurrence in Asia and South America, especially in Argentina, Bolivia, Colombia, Chile, Peru, Venezuela. In Brazil, in the basins of the Amazon River, Tocantins River, Araguaia River and São Francisco River (Figure 13), where the first record was recently confirmed by Barbosa et al. (2016).

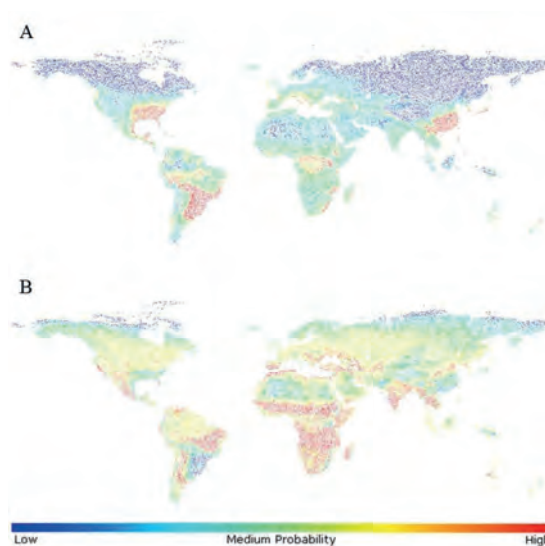


Figure 13. Maps of the means (A) and deviations (B) of the best potential distribution scenarios generated by the four types of algorithms (*Mahalanobis Distance, Domain, Garpand Maxent*). Source: Campos et al. (2014).

3. Impacts

Environmental and economic impacts of the invasion of *L. fortunei* are widely documented, affecting the multiple uses of water bodies and water and biodiversity.

3.1 Environmental Impacts

Environmental impacts of invasive golden mussel populations have been documented in the literature on different components of aquatic biota as well as freshwater habitats.

3.1.1 Aquatic Habitats

The water column, in addition to being occupied by large densities of larvae, suffers changes in transparency due to the intense filtration activity of adults, while hard substrates, previously occupied by epifaunal organisms, are completely covered by clusters of golden mussels (recruits and adults), which increases structural complexity and alters the quality of these habitats due to the large release of organic material through pseudofeces (UHDE et al., 2012, BOLTOVSKOY et al., 2015b; KARATAYEV et al., 2015). Root rot of emergent macrophytes can reduce the biomass of marginal vegetation, causing loss of habitat for foraging, reproduction and shelter of some species of fish and other organisms (MANSUR et al., 2003, 2004).

3.1.2 Aquatic Macrophytes and Periphyton

According to the review by Karatayev et al. (2015), the golden mussel *L. fortunei* indirectly affects the macrophyte and periphyton community, leading to an increase in biomass. These effects are due to changes in water quality, especially regarding the availability of nutrients, through the release of feces and pseudofeces (BOLTOVSKOY et al., 2015b).

During the first years of invasion in Lake Guaíba (panic phase), in the municipality of Porto Alegre (RS) area, it was possible to verify another type of impact on the macrophyte community, especially the emergent forms. The rushes, a typical reed plant formation, suffered a reduction in terms of plant cover. However, the macrophyte biomass was not monitored. *Mussel beds* of the golden mussel covered the rhizomes of the reed (*Scirpus californicus*, Figure 14), encrusting the base of the plant with an accumulation of pseudofeces, preventing oxygen circulation and causing the rhizomes to rot (MANSUR et al., 2003). During this same period, several fishermen reported a decrease in fishing resources due to the reduction of habitat for the reproduction of important species of

fish of commercial interest. The habitat of most of these fish consists of the reeds along the shores of Lake Guaíba. In another type of vegetation, the straw (Figure 16), the same effect of the population growth of the golden mussel was observed, with consequences on the vegetation cover and possibly the biomass, altering the coastal landscape of Lake Guaíba (MANSUR et al., 2012). Other macrophytes are also affected by the encrustation of the golden mussel, such as *Eichhornia crassipes* and *E. azurea* (CORREA et al., 2015), verified by incrustations in their stems.

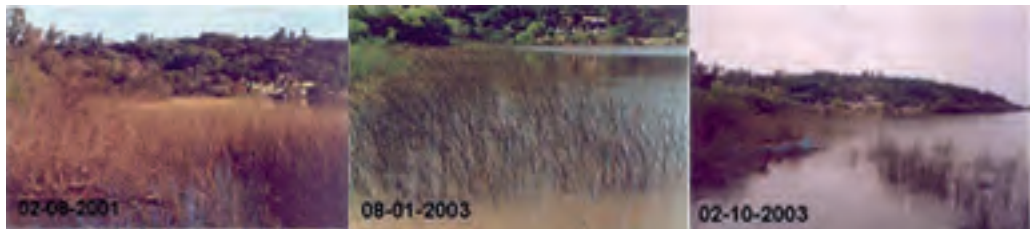


Figure 14. Loss of reed cover due to golden mussel incrustation on the shores of Lake Guaíba. Photo: MCD Mansur.



Figure 15. Golden mussel embedded in sarandi and straw rhizomes (*Cephalanthus glabratus*) on the banks of Lake Guaíba, forming continuous mattresses on the sandy substrate called *mussel bed*. Photo: MCD Mansur.

3.1.3 Planktonic Organisms

According to Karatayev et al. (2015), the golden mussel, like the mussel zebra finch and guaga mussel affect the phytoplankton and zooplankton communities, resulting in decreased densities and biomass, and changes in composition and productivity. The impact on the phytoplankton community structure tends to be severe, considering that the presence of zooplankton has no effect on the grazing of most groups of

phytoplankton, by the golden mussel, which feeds on both zooplankton and phytoplankton at the same time (FRAU et al., 2016).

Molina et al. (2012, 2015) associated selective grazing with decreased rotifer densities in several water bodies of the Middle Paraná River, Argentina, after the invasion of *L. fortunei*. Furthermore, according to the authors, the densities of golden mussel larvae normally exceed the sum of the densities of rotifers and zooplanktonic crustaceans during 8-9 months of the year, when the invader becomes dominant in the zooplankton. They also emphasize that the larvae would be competing with zooplanktonic species for food resources.

According to Boltovskoy et al. (2015b), studies on the consumption of cyanobacteria toxic effects of the golden mussel produce conflicting results. However, the authors stated that large populations of the golden mussel significantly increase the blooms of the *Microcystis* spp. by altering nutrient availability, size-selective filtration, promoting colony formation, and reducing grazing of toxic cells. Toxic blooms suppress mussel reproduction, probably by killing larvae.

As reported by Paolucci et al. (2010) and Paolucci and Thuesen (2015), larvae Plankton of fish are affected by the abundance of golden mussel veligers, which have become a predominant item in their diet, contributing significantly to their growth, especially of Characiformes and Siluriformes.

3.1.4 Benthic Fauna

According to the review by Karatayev et al. (2015), *L. fortunei* affects the benthic community marginal nica, leading to an increase in density and diversity of organisms, in the same way that was documented for the zebra mussel and the guaga mussel, by several authors. Both, *L. fortunei* and *Dreissena polymorpha*, do not have any effects on the deep zoobenthos, as they are not very abundant in these habitats or even absent.

Sylvester and Sardina (2015) compiled records from several authors on the effects effects of the golden mussel on the abundance, biomass and richness of zoobenthic organisms, related as follows: increased abundance of annelids (Polychaeta) and insect larvae of the orders Collembola, Coleoptera, Odonata (Coenagrionidae), Diptera (Ceratopogonidae, Psychodidae, Tabanidae, Tipulidae); increased abundance and biomass of Hydrozoa, Turbellaria, Tardigrada, Gastropoda molluscs (Cochiliopidae), microcrustaceans (Cladocera and Copepoda), aquatic mites (Arachnida) and insect larvae of the orders Diptera (Tanypodinae), Trichoptera and Ephemeroptera; increased abundance, biomass and richness of annelids (Hirudinea and Oligochaeta), and microcrustaceans (Amphipoda, Tanaidacea and Isopoda). The authors

also listed the effects on the reduction of abundance and biomass of Gastropoda molluscs (Planorbidae and Chylinidae) and on the abundance and richness of native freshwater bivalve molluscs (*Anodontites*, *Diplodon* and *Corbicula*). However, the authors did not report at which stage of the invasion process the effects on benthic organisms were observed. Probably, at different stages of population growth, during the invasion process, golden mussel populations generate divergent effects on the structure and composition of the benthic fauna. It is possible that in the panic phase, when the invader reaches extreme densities and expands rapidly, the effects are different from those observed when the invasive population reaches oscillatory equilibrium.

Encrustations on mollusc and bivalve shells have affected the structure and composition of the freshwater bivalve taxocenosis, being one of the threats to the conservation of some species. For example, in Rio Grande do Sul, bivalves such as *Leila blainvilliana*, *Diplodon deceptus*, *Diplodon hildae*, *Diplodon koseritzi* and the gastropod *Pomacea canaliculata* were recorded with golden mussel individuals embedded in their shells (MANSUR et al., 1999, 2003; SANTOS et al., 2012a; Figure 4). Epifaunal gastropods such as species of the genera *Gundlachia* spp. (Ancyliidae), *Potamolithus* spp., *Chilena* spp. also suffered from habitat reduction in Lake Guaíba, Rio Grande do Sul (MANSUR et al., 2008), and in Balneário Bagliardi, Argentina (DARRIGRAN; DAMBORENEA, 2009). In the Lower Jacuí River Basin, Kapusta and Freitas observed a greater richness of benthic organisms associated with macroclusters of golden mussels than in sandy bottom sediments.

3.1.5 Ichthyofauna

According to the review by Karatayev et al. (2015) on the parallel and con- between invasive Dreissenidae and the golden mussel, *L. fortunei* affects the ichthyofauna due to the greater availability of resources for both fish larvae and adult fish, as confirmed by a series of studies. As reported in the trophic relationships item, many fish have changed their feeding habits by acting in other trophic guilds, resulting in energy gains for these species that prey on the golden mussel. Harmful effects can be cited regarding fish species that, when feeding on mussels, without mouthparts adapted to crush the shells, suffer injuries near the anus due to the passage of the shells, which makes them susceptible to parasites.

3.1.6 Water Quality

Boltovskoy et al. (2015b) revealed through laboratory and field experiments that a series of long-term field data that golden mussel populations decrease the concentrations of particulate organic matter in the water column and increase the levels of ammonia, nitrate and phosphate, leading to an increase in the phosphorus/nitrogen ratio. It also causes

with increased water transparency, decreased seston, phytoplankton and primary productivity. According to Karatayev et al. (2015), the golden mussel affects the nutrient cycle in the aquatic environment by clarifying the water column.

3.1.7 Trophic Chain

According to Darrigran and Damborenea (2011), *L. fortuneis* considered an engineer species due to the following structural changes that they can cause in the ecosystem, due to their high filtration capacity: clarifying the water body, leading to increased light penetration, growth of submerged aquatic macrophytes and changes in the planktonic community; intensifying nutrient cycling, producing large quantities of organic material released by feces and pseudofeces, releasing them into the water column, which is later sedimented at the bottom of the water body, thus offering habitat and food resources for burrowing benthic organisms (infauna). They also mention structural changes due to shell clusters, due to the greater availability of hard substrate and increased substrate complexity, increasing mobile fauna and decreasing endemic epifauna; decreasing water flow due to the accumulation of shells. Still in relation to the production of clusters, the authors show potential impacts such as an increase in omnivorous fish, competition with native bivalves and degradation of reed roots (Juncaceae).

The effects on the complexity of the food chain are still very little known. cids, with different effects expected in lentic and lotic environments, due to the shorter and longer residence time of the water column and suspended materials, as well as aspects of hydrodynamics, such as current direction and vertical displacements of the water mass. However, it can be emphasized, based on the studies cited, that intensive grazing on planktonic communities reduces this food resource for planktonic fish species and fish larvae. In contrast, golden mussel larvae become an important energy resource for plankton consumers. Adult mussels release large amounts of feces and pseudofeces, increasing the ammonia, nitrate and phosphate content, making nutrients available to primary producers such as aquatic macrophytes, periphyton and phytoplankton. Despite considerable changes in the composition of phytoplankton, zooplankton and benthic organisms, many taxa experience an increase in abundance and biomass, as has also been documented for fish. However, it is not clear what damage is caused to the functioning of ecosystems invaded by bivalves or whether there is a trade-off between losses and gains, when different taxa in aquatic communities benefit from the invasion, acting in their original trophic guild or when they become part of a different guild.

According to Cataldo (2015), there is a lack of large-scale data on the effects

from the consumption of golden mussels by fish, but these are probably very significant, since the impacts are not restricted to malacophagous fish, but also to ichthyophagous fish that feed on them, as well as to detritivorous fish, which feed on sediment enriched by mussel feces and pseudofeces.

The synthesis of population effects of zebra mussels (*Dreissena* spp.) on the catrophic chain (excluding ichthyofauna), which were also observed for the golden mussel, carried out by Boltovskoy et al. (2015b), is briefly described below. At the bottom of the water body, the bivalve causes structural changes in the benthic habitats, through the formation of clusters, which increase food resources and shelter for the benthic invertebrate fauna, increasing the diversity and abundance of these organisms. Still in the bottom compartment, there is a high production of feces and pseudofeces rich in organic material. For the zebra mussel, the author reported the depletion of oxygen near the bottom, due to the decomposition of feces and pseudofeces, which may reduce the abundance of benthic macroinvertebrates, a fact not yet proven for the golden mussel. However, it is worth highlighting the rotting of the reed roots near the bottom sediment, where the accumulation of organic material released by *mussel beds* of the golden mussel reported by Mansur et al. (2003). In the water column, Boltovskoy et al. (2015b) reported effects of golden mussel populations on nutrient cycling, particularly on the increase in the phosphorus/nitrogen ratio, which may result in the formation and growth of colonial cyanobacterial blooms (*Microcystis* spp.), which consequently cause mortality of the invader's larvae, fish and birds, and degradation of water quality. Furthermore, it is reported that grazing on particulate matter and phytoplankton increases water transparency, which promotes the growth of submerged macrophytes and periphyton. The author also emphasizes that conflicting effects may occur for the phytoplankton, zooplankton and benthos communities, but that these effects still need to be proven.

3.2 Economic Impacts

The economic impacts mainly affect the electricity sector, due to the damage to equipment and reduction in generation efficiency. Other sectors affected include fishing, shipping, fish farming and water collection and treatment.

3.2.1 Power Generation and Reservoirs

The presence of the golden mussel results in structural damage to equipment. cooling, grates, filters (Figure 5), pumps, pipes, racks, grilles, screens, storage tanks, pump wells, water intake tunnels, submerged monitoring instrumentation, level gauges and concrete walls (SANTOS et al., 2012a; DARRIGRAN; DAMBORENEA, 2009, BOLTOVSKOY et al., 2015c). The interval between shutdowns

The maintenance costs for the Itaipu turbines were reduced after the golden mussel invasion, generating daily costs of US\$ 1 million (COLLYER, 2007). A 120 MW hydroelectric plant affected by the golden mussel, with three generating units, may have daily costs of R\$ 40,000.00 due to machine downtime, without taking into account the costs of equipment maintenance and removal of incrustations (NETTO, 2011).

3.2.2 Water Collection, Treatment and Distribution

Water collection faces major problems with clogged valves, pumps and grates (Figure 5), reducing the inflow. The distribution of water to the treatment plants is also impaired due to the clogging of the pipes. The invading organism can still enter the treatment plant (Figure 16), where, upon dying as a result of the treatment process, it generates organic residue that must be removed (MANSUR et al., 2003).



Figure 16. Corsan station, in Rio Grande do Sul, showing the colonization of tanks by the golden mussel and the residue removed. Photo: MCD Mansur.

3.2.3 Aquaculture

Aquaculture production in net cages is affected by molluscs growing in the nets and other metal surfaces of the tank (Figure 17), causing deterioration of the material, water quality, decreased flow within the tanks, increased weight of the tanks, drop in production, fish mortality and decreased useful life of the entire apparatus (OLI-VEIRA et al., 2014).



Figure 17. Golden mussel fouling in fish farming net cages in the Paranapanema Valley, São Paulo: A) golden mussel attached to the net cage screen, in this case, with a predominance of individuals larger than 5 mm; B) to the net cage floats; C) deposits of mussel shells on the edge of the reservoir, close to the fish farming area (Photos: Márcia D. Oliveira); D) golden mussel shells accumulated at the net cage cleaning site (Photo: Luiz Ayroza). Source: Oliveira et al. (2014).

3.2.4 Navigation and Waterways

Structural damage is also seen in the locks both on metal surfaces and in concrete. Furthermore, boats are an important vector of dispersion in these waterways (Figure 18). The transposition of basins increases the connectivity between invaded and non-invaded water bodies, increasing the risk of dispersion via water transport, in which the invasive bivalve can be transported either fixed to the hull of vessels or in water storage tanks (BOLTOVSKOY et al., 2015c).



Figure 18. Locks in the Jacuí River Basin, Rio Grande do Sul, showing natural and artificial substrates contaminated by golden mussels, as well as the hulls of vessels. Photos: MCD Mansur.

3.2.5 Fishing

According to Moraes (2012), in the coastal lagoons of the northern coast of Rio Grande do Sul, In the Tramandaí River Basin, fishermen reported in interviews that the golden mussel caused the decline of the reeds (Juncaceae), which constitute a nursery for local fish species, in decline, by attaching themselves to the roots of the macrophyte. In addition, they reported that the mussels decompose when the lakes' water levels drop, releasing a foul odor.

3.2.6 Irrigation

Of the potential impacts of the golden mussel, irrigation is cited by most by the authors (SANTOS et al., 2012a; DARRIGRAN; DAMBORENEA, 2009). However, there are no known case studies that record this impact and measure its magnitude. During an inspection of water collection systems on the Jacuí River in Rio Grande do Sul, golden mussels were detected in irrigation and drainage channels in the Passo Raso area in Triunfo (Pereira, unpublished data). However, no significant damage to irrigation activities was observed. The abundance of golden mussels in these channels was low, with a few individuals found attached to some wooden trunks located on the banks of the channels. Boltovskoy et al. (2015c) reported this problem only for Japan and China.

3.2.7 Tourism

The accumulation of shells on freshwater beaches (Figure 4I) hinders circulation of bathers, which can result in accidents such as cuts to the feet, when trampling on

shells of dead animals, both in and out of the water (SANTOS et al., 2012a). This situation can affect tourism on beaches where the mollusk population growth is significant. This situation generally occurs after the sudden lowering of the water level, during dry and low tide months, when the bivalves are exposed to the sun for a long period on the banks, leading to the mortality of clustered individuals that accumulate on the sediment.

4. Prevention

In 2004, the International Maritime Organization (IMO) established guidelines for prevent bioinvasions through ocean navigation. One of the guidelines consists of the oceanic exchange of ballast water. The water collected in coastal regions is replaced by ocean water, with a volumetric efficiency of 95%, making the survival of organisms with invasive potential unfeasible, due to changes in water quality parameters. The exchange must be carried out at a distance greater than 200 nautical miles from the coastline of the port of destination, in places with a depth equal to or greater than 200 m. Another guideline consists of the preparation of a Ballast Water Management Plan for all ports and ships, and ships must also treat their ballast water using physical methods or biocides (FERNANDES; LEAL-NETO, 2009). The International Convention for the Control and Management of Ships' Ballast Water and Sediments came into force on September 8, 2017, when the target of 30 countries was reached, which together represent at least 35% of the gross tonnage of the world merchant fleet. In Brazil, the text had already been approved via Legislative Decree No. 148/2010. Controlling ballast water is a preventive measure against new invasions and the spread of the species on the continent. The standard that guides the management of ship ballast water is Normam-20/DPC, from the Brazilian Navy.

Preventive actions are of utmost importance to contain the spread of the virus. golden finch to uncontaminated river basins in Brazil. These measures should involve different actors in society and be implemented prior to the detection of the invasive species, with the purpose of alerting to the main vectors of dispersion, which are mostly caused by human activities (DARRIGRAN et al., 2012) (Table 4). Furthermore, according to these authors, prevention actions should integrate knowledge about the biology and ecology of the invasive species, management methods, public policies, legislation and regulations, and economic, philosophical and social issues.

Fillipo et al. (2012) documented a series of preventive actions in the states of São Paulo, Minas Gerais, Goiás, Mato Grosso and Mato Grosso do Sul, integrated, using various means of communication such as radio, television, newspapers and the Internet. The actions were carried out at large fishing festivals and regional parties, with the help of local authorities, educators, managers and artists. In addition, a series of lectures and educational materials were distributed to the public education network. In addition to informative material,

T-shirts, caps, pens and stickers, with a toll-free telephone number, aiming to facilitate the communication of new records of occurrence of the golden mussel. This set of pioneering and integrated actions is extremely effective and serves as a model to be followed throughout the national territory, especially in the basins to the north and northeast of the country, which are geographically close to areas invaded by the golden mussel.

Information materials provided by Furnas and Cemig clarify procedures for disinfecting and removing fouling from fishing gear and vessels. However, these procedures lack standardization and emergency regulation so that they can be adopted on a large scale in Brazilian waters, with the aim of preventing the spread of the invasive species.

5. Eradication

There are no known cases of eradication of the golden mussel after detection and dispersion of the species in countries in South America and Asia. The attributes of this invasive species favor rapid dispersion between river basins by natural and artificial vectors. It is a priority to establish measures and procedures to minimize the risk of dispersion of the species to areas not yet invaded. In cases where control is possible, for example in industrial plants, it is necessary to establish guidelines and good practices to mitigate possible impacts on the environment. Currently, there are no methods available for eradicating the golden mussel, which makes this task very difficult even in places where invasion has begun (DARRIGRAN; DAMBORENEA, 2009).

6. Monitoring

Monitoring programs are intended to obtain population data on the dispersion of invasive bivalves into new areas, enabling water resource users to prepare themselves to face the problem of fouling in their facilities (CLAUDI; MACKIE, 1994). In addition, they provide subsidies for environmental agencies to plan their management actions.

Monitoring of invasive molluscs can be divided into three levels, according to with its objectives, according to Mackie and Claudi (2010): 1) monitoring for early detection of the invasive species, in areas where there are no records yet, but with limnological conditions, dispersion attributes and risk analysis results that justify the effort; 2) temporary post-invasion monitoring, in a hydrographic system, aiming to understand the levels of infestation at different times of the year, the reproduction period, growth rates, longevity and settlement, with the objective of subsidizing actions

control in industrial systems and other human activities; 3) long-term monitoring, aiming to develop studies of the spatial-temporal distribution along large watercourses and their hydrographic basins, to determine population variations and their relationships with environmental variables between different compartments of the water body, characterized by different depth levels, substrates, water quality, etc. Two other objectives of monitoring programs were recognized by Claudi and Mackie (1994): 4) to determine the effectiveness of control programs; and 5) to minimize the costs of control programs.

6.1 Protocols

According to Claudi and Mackie (1994), there are two types of monitoring: 1) *mainstream*, which consists of conventional monitoring carried out in the water body, in which collections are made directly from surface waters such as lakes, rivers and reservoirs; 2) *sidestream*, which can be called external lateral flow monitoring, with sampling being carried out in a device coupled to a water distribution or circulation system in an industrial or water treatment plant. The monitoring methods reported below are of the type *mainstream*.

6.1.1 Qualitative Larvae Test (PCR)

Veliger larvae are an important attribute of invasion success, therefore, Ideally, they should be detected as soon as they are introduced into a new area, survive the transport of water, in the ballast of ships or in reservoirs (cisterns and nurseries) of small vessels, initially occurring in small quantities. Qualitative methods only allow the assessment of the presence of larvae, and it is not possible to determine the population density, but they are very useful for detecting the species in areas where the population is incipient or is in a lapse phase (resting). The qualitative method for detecting larvae in plankton was developed by Pie et al. (2006) and is described in both the book organized by Darrigran and Damborenea (2009), published by AES Tietê, and in the book organized by Mansur et al. (2012), published by Furnas, both with texts in Portuguese. This method is based on simple molecular biology procedures (TSCHÁ et al., 2009). Although it is more sensitive than the examination of larvae by optical microscopy, this method is more expensive and requires a basic molecular biology laboratory with trained technicians. The laboratory procedures are described in detail in Tschá et al. (2009 and 2012) and basically consist of: collecting a zooplankton sample, vacuum filtration of the sample, extraction of zooplankton DNA, amplification based on the DNA polymerase chain reaction (*Polymerase Chain Reaction*(PCR)) using the *primers* (LIMNO.COIR1 and LIMNO.COIF1), electrophoresis and visualization of the results in agarose gel, allowing the detection of the presence or absence of *L. fortunei*.

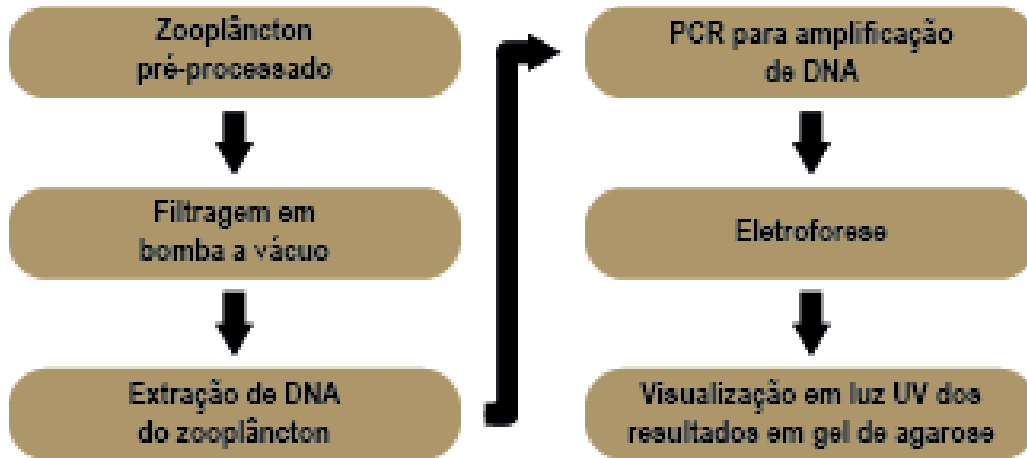


Figure 19. Steps of the PCR protocol for detecting the presence and absence of golden mussel larvae. Source: Tschá et al. (2012).

6.1.2 Quantitative Larvae - Optical Stereomicroscopy

The quantification of larvae is extremely necessary in order to understand to monitor reproductive dynamics, monitor population growth, substrate recruitment and evaluate the effectiveness of population control methods. The quantitative method using optical microscopy is described in Santos et al. (2012b) and consists of filtering a known volume of water (1,000 L) using a plankton net with a 30 µm mesh opening, with the aid of a suction pump, resulting in the concentration of the sample taken from the water column. Subsequently, quantification is performed using a stereoscopic microscope to calculate densities expressed as larvae.⁻³. Although it presents lower sensitivity than molecular methods, the cost of this method is lower; however, it requires a trained technician to recognize the larval stages of the golden mussel (Figure 3), which were described by Santos et al. (2005), and distinguish them from other larvae of native and invasive Cyrenidae bivalves, which are described in Mansur et al. (2012).



Figure 20. Collection of plankton samples for quantification of larvae of *L. fortunei*. A) filtration in plankton net, B) plankton net, C) suction pump, D and E) removal of the sample from the net and F) fixation of the sample in 80% alcohol. Source: Santos et al. (2012b).

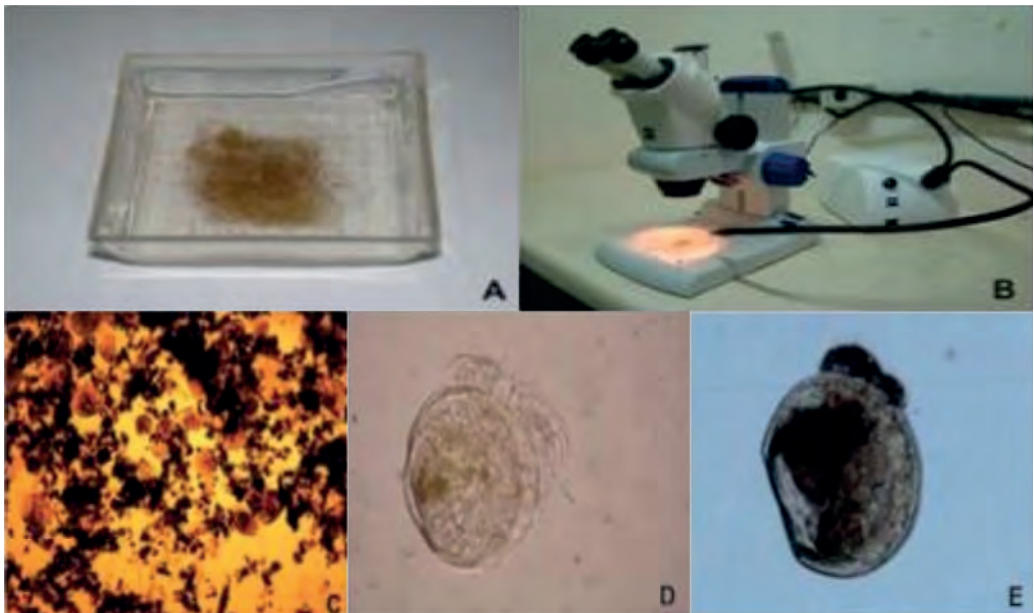


Figure 21. Quantification of golden mussel larvae. A) sample on counting plate, B) stereomicroscope with sample under examination, C) sample being observed, D and E) larvae displaying the veil. Source: Santos et al. (2012b).

6.1.3 Larval Quantification – Optical Stereomicroscopy with Polarized Light

This method is based on a phenomenon caused by light (called birefringence), which, due to the shape of the larval shell, makes it possible to view the organisms under a stereomicroscope, through the incidence of polarized light, and highlights the larvae against a black background, even in samples rich in suspended material (SILVA et al., 2016).

6.1.4 Quantitative Larvae – Real-Time PCR

The quantitative method of detecting larvae in plankton using the reaction in ca- Quantitative real-time polymerase chain reaction (qPCR) is extremely sensitive (ENDO; NO-GATA, 2012) and requires slightly more sophisticated equipment, which makes its use more expensive when compared to the qualitative molecular method and the quantitative method by optical microscopy. However, according to the authors, it is a very effective method for monitoring the bioinvasion process and confirming new records in areas with low larval density or with a lack of technicians trained to recognize the larval stages of the golden mussel. The laboratory procedures are described in detail in the book distributed by Furnas, in the chapter by Endo and Nogata (2012). These basically consist of: collecting a zooplankton sample, fixing it in 99.5% alcohol and storing it at 4 °C until the time of analysis, performing qPCR amplification, verifying the specificity of the qPCR amplification and estimating the number of larvae *L. Fortunei*, plotting the Ct values on the standard curve.

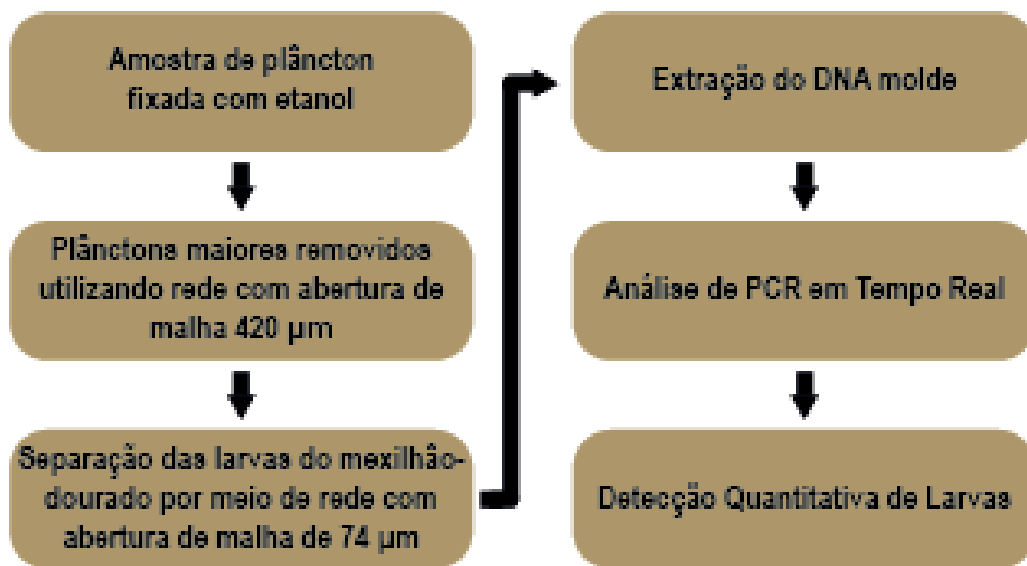


Figure 22. Steps of the qPCR protocol for detecting the presence and absence of golden mussel larvae.

Source: Endo and Nogata (2012).

6.1.5 Larval Quantification – Automated Particle Analysis

A sample is displaced by a flow generated by a suction pump that is coupled to a microscope and this to a computer equipped with an image capture program, which automatically explores the entire sample in search of particles of interest, in this case, golden mussel larvae (SILVA et al., 2016).

6.1.6 Recruits and Adults – Scrapers

When it reaches the benthic phase, the golden mussel colonizes hard substrates. Therefore, scraper shells are a good option for rapid sampling and can be used for quantitative sampling of concrete walls and other smooth surfaces in which mussels have become encrusted. Pereira et al. (2012b) describe the equipment as a crescent-shaped aluminum or stainless steel container with a straight, sieved bottom and threaded onto a handle; the straight end of the crescent has a serrated edge, which is used to scrape encrusted surfaces; the sampled area is delimited considering the width of the sampler and the distance perpendicular to the width of the sampler covered in the scraping process.



Figure 23.Scraper shell for collecting golden mussel incrustations. Source: Pereira et al., 2012b.

6.1.7 Quantification of Recruits and Adults – Bottom Search

Bottom-seeking samplers can be used for sampling mussels. golden finch in the zoobenthic community of the bottom of rivers and reservoirs (TAKEDA; FUJITA, 2012; PEREIRA et al., 2012b). The various models and considerations on the advantages and disadvantages of using these equipments are described in detail in Pereira et al. (2012b).

6.1.8 Quantification of Recruits and Adults – Artificial Substrates

Artificial substrates are artifacts that imitate characteristics of the natural habitat of benthic organisms. Two types of artificial substrates have been widely used in Brazil in monitoring programs and research projects related to the golden mussel: three-dimensional wooden structures in the shape of an “X” and ceramic substrates, bricks or small tiles. These substrates have been widely used in studies carried out by environmental agencies and hydroelectric power concession companies. The method consists of suspending the substrates in the water column and monitoring the colonization of recruits monthly and of adults every 3 or 4 months. The methods based on artificial substrates are described in detail in Pereira et al. (2012b).

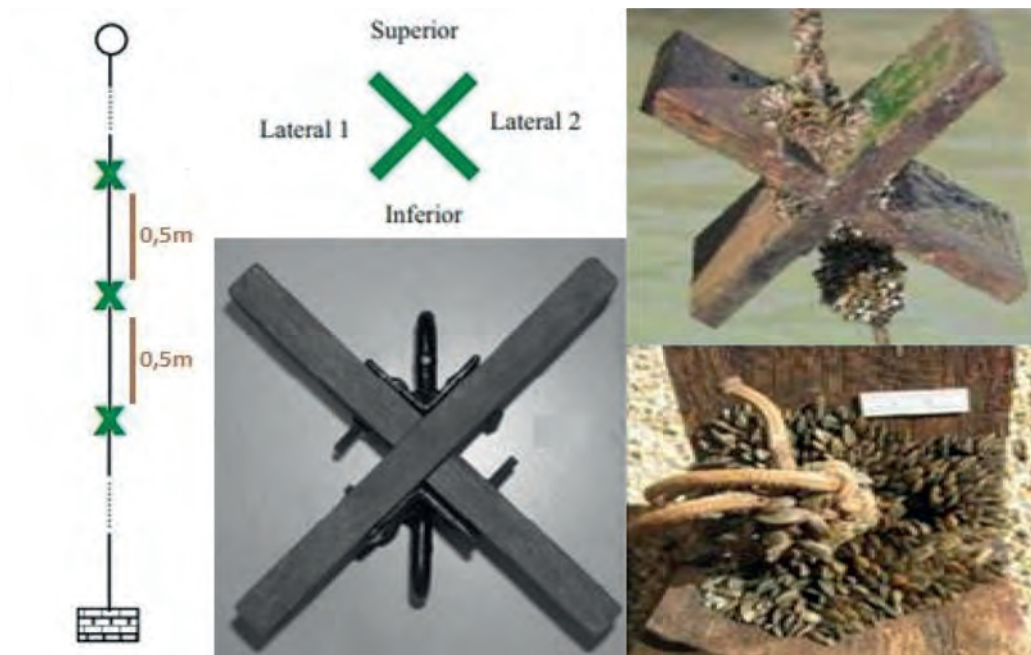


Figure 24. Artificial wooden substrate in the shape of an “X” for sampling recruits and adults of the golden mussel. Source: Pereira et al. (2012b).

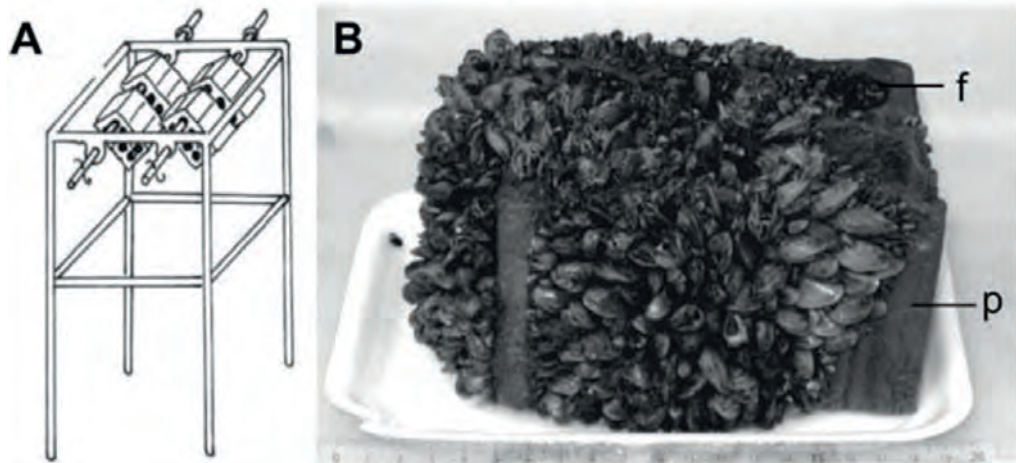


Figure 25. Ceramic bricks used as an artificial substrate for sampling recruits and adults of the golden mussel. Source: Pereira et al. (2012b).

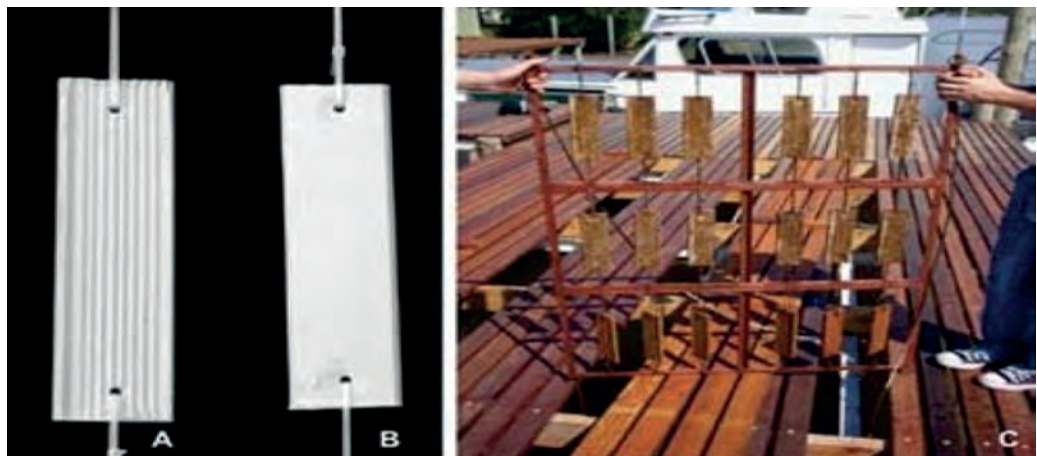


Figure 26. Ceramic tiles used as an artificial substrate for sampling recruits and adults of the golden mussel. Source: Pereira et al. (2012b).

7. Population Control

According to Darrigran and Damborenea (2009), population control of the golden mussel The treatment is mainly focused on industrial facilities and can be planned through two approaches: 1) proactive treatment, in which doses of molluscicides are applied to control the settlement of larvae, through intermittent, continuous or semi-continuous applications. This approach is adopted from the beginning of the period of larval release into the plankton; 2) reactive treatment, in which the target is the adult individuals. It can be applied at the end of a reproductive season or periodically. Both approaches require population monitoring to monitor population fluctuations of larvae and adults. The population control of invasive bivalves can be carried out by chemical, physical and biological control agents.

In Brazil, Temporary Special Registration (RET) is mandatory for technical products, premixes, pesticides and similar products intended for research and experimentation. Temporary Special Registration is, according to Decree No. 4,074/2002, the exclusive act of a competent federal agency intended to grant the right to use a pesticide, component or similar product, for specific purposes in research and experimentation, for a specified period of time, and may grant the right to import or produce the quantity necessary for research and experimentation.

It is up to the Ministry of Agriculture, Livestock and Supply to grant registration, including the RET, of pesticides, technical products, premixes and the like for use in the sectors of production, storage and processing of agricultural products, in planted forests and in pastures, meeting the guidelines and requirements of the Ministries of Health and the Environment (Decree No. 4,074/2002). The Ministry of Health is responsible for granting registration, including the RET, of pesticides, technical products, premixes and the like, intended for use in urban, industrial, domestic, public or collective environments, for water treatment and for use in public health campaigns, meeting the guidelines and requirements of the Ministries of Agriculture and the Environment. Finally, it is up to the Ministry of the Environment, through Ibama, to grant registration, including RET, of pesticides, technical products and premixes and the like, intended for use in water environments, in the protection of native forests and other ecosystems, meeting the guidelines and requirements of the Ministries of Agriculture, Livestock and Supply and the Ministry of Health.

It is important to highlight the definitions set out in Law No. 7,802, of July 11, 1989, which provides for research, experimentation, production, packaging and labeling, transportation, storage, marketing, commercial advertising, use, import, export, final destination of waste and packaging, registration, classification, control, inspection and supervision of pesticides, their components and the like, and provides other measures. According to Law No. 7,802/1989, pesticides are considered to be products

and agents of physical, chemical or biological processes, intended for use in the sectors of production, storage and processing of agricultural products, in pastures, protection of native or planted forests, and other ecosystems, and also in urban, water and industrial environments, whose purpose is to alter the composition of flora or fauna, in order to preserve them from the harmful action of living beings considered harmful. According to art. 3, pesticides, their components and the like may only be produced, exported, imported, marketed and used if previously registered with a federal agency, in accordance with the guidelines and requirements of the federal agencies responsible for the health, environment and agriculture sectors.

In view of the above, all forms of control of the golden mussel that fall within the scope of rem in the definition provided in Law No. 7,802/1989 must be registered so that they can be produced, exported, imported, marketed or used in the Country, following the indications provided for in the law and in its Regulatory Decree No. 4,074, of January 4, 2002.

According to Decree No. 4,074/2002, it is the responsibility of the Ministry of the Environment, especially to Ibama:

- I. Evaluate pesticides and similar products intended for use in aquatic environments, in the protection of native forests and other ecosystems, regarding the efficiency of the product;
- II. Carry out an environmental assessment of pesticides, their components and similar products, establishing their classifications according to their potential for environmental hazard;
- III. Carry out a preliminary environmental assessment of pesticides, technical products, premixes and similar products intended for research and experimentation;
- IV. Grant registration, including RET, of pesticides, technical products, premixes and the like, intended for use in water environments, in the protection of native forests and other ecosystems, in compliance with the guidelines and requirements of the Ministries of Agriculture, Livestock and Supply, and Health.

If it is a product based on a microbiological agent, it will fall under in Annex III of INC No. 25/2005, which contains a list of specific products (such as biological control agents, microbiological agents, with the exception of those obtained through genetic engineering techniques). To request Temporary Special Registration, the applicant

must forward the provisions of Annex II of the aforementioned instruction, which contains the Technical Report for products listed in Annex III. The legislation governing the procedures to be adopted when requesting RET is Decree No. 4,074 of January 4, 2002, and Joint Normative Instruction No. 25 of September 14, 2005.

According to INC n° 25/2005: In addition to the provisions of art. 7 of this Instruction According to the regulations, this document must be signed by the landowner and the applicant, or by their legally constituted representatives. Once the RET has been granted, and after the product's trial period, its registration may be requested. In this case, in the case of a microbiological control agent, the provisions of INC No. 03/2006 must be followed, which establishes procedures to be adopted for the purpose of registering microbiological agents used to control a population or biological activities of another living organism considered harmful. In this case, all documents and studies related to the aforementioned regulation must be submitted at the time of the registration request (which must also be made to the three agencies). Ibama Normative Instruction No. 5, of August 26, 2016, must also be considered. It establishes the procedure for the application for registration or temporary special registration for biological agents or products based on microbiological agents, exotic or without proof of natural occurrence in the country, intended for the biological control of pests and diseases, until criteria are established for assessing the risks of their introduction into Brazilian territory. This standard must be considered for the use of products based on biological control agents in terrestrial environments or in Brazilian hydrographic systems.

7.1 Physical Control

Physical control can be done by mechanical removal, high pressure pumps, sound waves, ultraviolet radiation and by means of anti-fouling coatings, without biocides (ZURITA, 2012). Methods considered mechanical, such as scraping, filtration, blasting and turbulence, are exempt from registration. However, physical methods, such as those involving ultraviolet radiation, ultrasound, electric current and magnetic field, require registration with Ibama, in compliance with the guidelines and requirements of Law No. 7,802/1989 and complementary standards, in addition to authorization for use issued by the competent environmental agency, considering the location of the area or enterprise where the control of the invasive organism is intended.

It is recommended that you consult the Ibama website to verify whether the physical process intended for controlling the golden mussel has a current registration (<http://ibama.gov.br/agrotoxicos/registro-de-agrotoxicos-de-uso-nao-agricola#listaregistrados>; <http://www.ibama.gov.br/agrotoxicos/registro-emergencial-de-agrotoxicos-e-afins#listadeprodutos>; <http://www.ibama.gov.br/agrotoxicos/ret>).

7.1.1 Scraping

One form of physical control is mechanical removal by scraping, using robots or divers, the latter being widely used by public water collection and treatment companies in Rio Grande do Sul (Dmae and Corsan) and Paraná (Sanepar). However, this type of control damages the surfaces of the materials and can open the way for corrosion, in addition to the high cost and time required for cleaning, which requires equipment shutdown.

7.1.2 Filtration

Claudi and Oliveira (2015a) suggest sand filters or self-cleaning mechanical filters to remove mussel larvae in industrial plants, using a pore size of 150 μm . According to the authors, experiences in Canada were satisfactory for the removal of veligers from invasive bivalves in that country. This is a form of proactive treatment.

7.1.3 Sandblasting

This is a form of reactive treatment, in which physical control can be done by blasting, using high-pressure pumps. It is an excellent resource for removing mussels stuck to equipment, metal surfaces and concrete. It has been recommended by Oliveira et al. (2014) for cleaning net cages used in fish farming. The measure is efficient, but requires care in the disposal of the removed mussels, which must be discarded on land to avoid increasing organic matter and, consequently, the biochemical demand for oxygen in the aquatic environment.

7.1.4 Ultraviolet Radiation

Ultraviolet radiation treatment is a form of proactive treatment. To *D. polymorpha*, a dosage of 100 mWs/cm^2 inhibits the settlement of this Dreissenidae in North American environments (CLAUDI; OLIVEIRA, 2015a). Physical control by ultraviolet radiation was tested in a pilot unit by Santos et al. (2012c), with raw water. The authors found a dose of 324 mWs/cm^2 to inactivate 50% of larvae in a flow range between 1400 and 4200 L/h and 781 mWs/cm^2 to reach 100%. However, Claudi and Oliveira (2015a) point out that the experiment cited did not consider the absorbance of the water used, did not evaluate delayed mortality and did not measure the real dose, estimating it based on the lamp manufacturer's specifications, resulting in overestimated doses. Lower doses were found by Perepelizin and Boltovskoy (2014): 149 mWs/cm^2 to cause 100% mortality at 25.8 °C and 103 mWs/cm^2 to cause 100% mortality at 23 °C. According to Claudi and Oliveira (2015a) and Souza et al. (2000), the high content of suspended solids in South American rivers is one of the major limitations to the application of this method.

7.1.5 Magnetic Field

This technology is one of the most efficient in inhibiting settlement, causing mortality in at least a fortnight (CLAUDI; OLIVEIRA, 2015a).

7.1.6 Electric Current

Katsuyama et al. (2005) found that a voltage of 7kV immobilized 80% of the larvae in flow conditions, making settlement impossible.

7.1.7 Ultrasound

Ultrasound is a form of proactive treatment. Santos et al. (2012d) tested the acoustic effects of ultrasound on veligers in the laboratory and found a dose of 44kWs/L (>100kHz) to cause 30% mortality in *L. fortunei*, while 100% mortality was verified at a frequency of 20kHz. However, very well-planned acoustic insulation is necessary to keep this equipment working in the plant, without affecting the occupational health of workers and without causing problems to the equipment structures.

7.1.8 Turbulence

Turbulent flow influences the distribution of golden mussels in river basins. (CAMPOS et al., 2016), and the absence of fouling problems in certain reservoirs can be attributed to the high flow. According to Claudi and Oliveira (2015a), turbulent flow is generated by destabilization of the flow, when moving over a highly rough surface, forming unstable vortex patterns. Turbulent flows created by the passage of water over perforated plates can cause 80% mortality after 5 minutes (XU et al., 2013). The settlement of planktonic larvae is difficult at flow velocities greater than 1.3 m/s (MATSUI et al., 2002); however, it is difficult to manage the velocity in most industrial equipment (MACKIE; CLAUDI, 2010). In Brazil, there are no known successful experiments.

7.2 Chemical Control

Claudi and Oliveira (2015b) compiled chemical control strategies for the mussel golden mussels tested to date, listing 24 chemical compounds (Table 5), their effects, lethal doses and mortality percentage. Of the 88 toxicity assessments of chemical agents for controlling golden mussels compiled by the authors, the majority were carried out by exposing adults (91%), compared to only 9% of these studies being

carried out by exposing the larval stage. Controlling settlement is indeed the greatest challenge, as it interferes with the life cycle of the invading organism, but experiments with the larval stage are more laborious and require greater care regarding the accuracy of the results, and delayed mortality must be assessed (CLAUDI; OLIVEIRA, 2015b). Also according to this survey, 71.6% of the experiments were carried out in a static regime and only 28.4% were carried out in a continuous flow regime. The continuous flow experiments present more realistic simulations regarding the dilution and bioavailability of the control agents, as well as the production of byproducts, which can confer toxicity to biota and humans. Most chemical control agents require very high dosages, posing risks to the aquatic environment and biota, many of which lack ecotoxicological studies that deepen knowledge about ecotoxicity.

In Brazil, only two products received emergency registration on a temporary basis. Temporary, in accordance with Decree No. 4,074/2002, for exclusively industrial use, they are: MXD-100 (Ibama Normative Instruction No. 17, of October 21, 2015) and Sodium Dichloroisocyanurate (Ibama Normative Instruction No. 18, of October 21, 2015). All other products mentioned below, in this item, are not regulated and are not permitted for industrial use or in the natural environment. The use of products must strictly follow the indications for use included in the registration, emergency registration or temporary special registration, intended for research or experimentation, and cannot, therefore, be used in environments not provided for in the respective registration obtained from Ibama, meeting the guidelines and requirements of Law No. 7,802/1989 and complementary standards, and authorization for use issued by the competent environmental agency (Conama Resolution No. 467/2015), considering the location of the area or enterprise where it is intended to control the invasive organism.

On the Ibama website, you can check whether a chemical product or The physical process for controlling golden mussels has a current registration (<http://ibama.gov.br/agrotoxicos/registro-de-agrotoxicos-de-uso-nao-agricola#listaregistrados>; <http://www.ibama.gov.br/agrotoxicos/registro-emergencial-de-agrotoxicos-e-afins#listadeprodutos>; <http://www.ibama.gov.br/agrotoxicos/ret>).

7.2.1 Coatings

Antifouling coatings with different biocide formulations based on in copper and zinc were tested by Bergmann et al. (2010a, 2010b) for the control of the golden mussel. However, they did not show satisfactory results, since the growth of biofilms on the test specimens coated with these paints was followed by incrustations of the golden mussel. In addition, they have a very short useful life. Coutinho et al. (2012) evaluated the toxicity of nine commercial coating formulations (paints),

widely used in the naval industry to control fouling, with high toxicity for microcrustaceans *Daphnia similis* in three formulations, medium in two formulations and low in four formulations. Of the coatings with low and medium toxicity, five formulations were chosen, as they showed anti-adhesion efficiency to the golden mussel in laboratory experiments for application on boats used in navigation. The experiments resulted in the selection of three antifouling coatings with low toxicity to non-target organisms, which are listed below, in decreasing order of efficiency: paint *Copper Bottom*60, from the manufacturer *Sherwin Williams*, *Aemme Long Life Plus* and *Revrans* AF LCL 870. In studies of macroinvertebrate colonization in test specimens coated with the same paints, Fujita et al. (2015) found that the coatings *Copper Bottom*60 and *Aemme Long Life Plus* also inhibited the colonization of macroinvertebrates in the substrates, affecting the richness and density of taxa. The release of biocides from paints depends on a series of factors such as the solubilization of the biocide, which depends on the contact of the coating with water, determined by speed, friction, pH that determines leaching, temperature, among others (CAPRARI, 2006).

7.2.2 pH adjustment

pH adjustment using sodium hydroxide (unregulated, no permit from use) was tested by Calazans and Fernandes (2012) and Montresor et al. (2013) (Table 5). Due to the wide pH tolerance range (4-11, after five days of exposure) presented by *L. fortunei* (PEREIRA, 2014), any adjustment necessary to control fouling would result in values that are not appropriate for maintaining aquatic life, considering that classes I, II and III of Conama Resolution No. 357/2005 establish the pH standard with minimum and maximum values of 6 and 9, respectively. pH adjustment comprises a form of proactive treatment (CLAUDI; OLIVEIRA, 2015b).

7.2.3 MXD-100

By means of Ibama Normative Instruction No. 17, of October 21, 2015, the product was regulated by emergency registration only for use in cooling systems of hydroelectric plants, with the objective of creating an inappropriate microenvironment for the fixation of golden mussel larvae, aiming to prevent infestations of the mollusk. The emergency registration of MXD-100 was canceled due to insufficient data and failure to present the mandatory tests for definitive registration. Ibama Normative Instruction No. 17 provides the following guidelines: the dose may vary between 1 mg/L and 7 mg/L, according to the size of the ducts and the level of incrustation; the active ingredient content to be achieved to ensure the effectiveness of the treatment varies from 0.08 mg/L to 0.56 mg/L, thus remaining in the aqueous system for 10 minutes, every 8 hours, totaling 30 minutes daily; concentrations of MXD-100 greater than 2 mg/L may only be used for a maximum period of 30 days

and after this period they must be reduced to 1 to 2 mg/L; the application of the product, without prior dilution, must be done using a control system coupled to the cooling system of the generating units of the hydroelectric plant, with the product being dosed automatically, by a dosing pump, according to the established schedule and the maintenance of the concentration of the product in the monitored system; monitoring of the effluent to be released into the water body must be carried out, in addition to other requirements that may be established by the competent environmental agency, under the terms of Conama Resolution No. 467/2015 and Conama Resolution No. 430/2011.

The active ingredients of MXD-100 are Didecyl Dimethyl Ammonium Chloride (CAS No. 7173-51-5) and Alkyl Amido Propyl Dimethyl Benzyl Ammonium Chloride (CAS No. 124046-05-5) (IBAMA, 2015a). Effective concentrations of MXD-100 to cause mortality of *L. fortunei* were evaluated by several authors (PEREYRA et al., 2011; MATA et al. 2013; MONTRESOR et al. 2013) (Table 5).

Table 5. Summary of test results of chemical control agents tested with *Limnoperna fortunei*. Toxicant [trade name]: (1) 2,5'-dichloro-4'-nitrosalylcarbonylde (active ingredient: 70%) [Bayluscide WP70]; (2) Chlorine dioxide; (3) Diallyldimethylammonium chloride polymer (active ingredient: 40%) [Veligon TL-M]; (4) Didicly dimethyl ammonium chloride (active ingredient: 50%) [H130M]; (5) N-Alkyl dimethylbenzylammonium chloride (active ingredient: 50%) [Clam-Trol CT-2]; (6) N-Alkyl dimethylbenzylammonium chloride (active ingredient: 50%) [Spectrus CT1300]; (7) Poly(oxyethylene(dimethylimino)ethylene(dimethyliminio)ethylene) dichloride [Bulab 6002]; (8) Poly(oxyethylene(dimethyliminium)ethylene(dimethyliminium)ethylene) dichloride [Bulab 6002]; (9) Polydiallyldimethylammonium chloride (microencapsulated); (10) Potassium chloride; (11) Potassium chloride (microencapsulated); (12) Quaternary samples + tannin extracts [MXD- 100]; (13) Tannins *Schinopsis balansae* 70% [Ecotec-L]; (14) tannins *Schinopsis balansae* 74% [Ecotec-UA]; (15) tannins *Schinopsis balansae* 86.5% [Ecotec-MC]; (16) Sodium chloride; (17) Sodium dichloroisocyanurate; (18) Sodium hydroxide; (19) Sodium hypochlorite; (20) Total ammonia; (21) Trichloroisocyanuric acid; (22) United ammonia (NH₃-N); (23) Potassium permanganate; (24) Copper sulfate. Source: Claudi and Oliveira (2015b).

Agent of control	LC50 or % Mortality	[Post-] Exhibition [exposition] (h)	No. of concentrations tested (Range, ppm)	Size (mm)	Temp. (°C)	Conditions of Test	Reference
1	1.0 ppm	48 [24-264]	7 (0.25-8)	A (15-25)	15	Static	Cataldo et al. (2003)
1	0.8 ppm	48 [24-264]	7 (0.25-8)	A (15-25)	20	Static	Cataldo et al. (2003)
1	0.3 ppm	48 [24-264]	7 (0.25-8)	A (15-25)	25	Static	Cataldo et al. (2003)
2	427.6 ppm	48 [48]	14 (1-800)	A (15-25)	25	Static	Cataldo et al. (2003)
3	3.88 ppm	720 [0]	5 (2-50)	THE	20-22	Static	Boltovskoy and Cataldo (2003)
3	815.04 ppm	264 [0]	5 (0.5-10)	L	20-22	Static	Boltovskoy and Cataldo (2003)
4	0.56 ppm	720 [0]	5 (0.5-10)	THE	20-22	Static	Boltovskoy and Cataldo (2003)
4	1.03 ppm	264 [0]	5 (0.5-10)	L	20-22	Static	Boltovskoy and Cataldo (2003)
4	2.9 ppm	48 [24-264]	5 (0.5-10)	A (15-25)	15	Static	Cataldo et al. (2003)
4	1.7 ppm	48 [24-264]	5 (0.5-10)	A (15-25)	20	Static	Cataldo et al. (2003)
4	0.8 ppm	48 [24-264]	5 (0.5-10)	A (15-25)	25	Static	Cataldo et al. (2003)
5	2.43 ppm	36 [252]	5 (1-3)	THE	20-22	Static	Boltovskoy and Cataldo (2003)

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Agent of control	LC50 or % Mortality	[Post-] Exhibition [exposition] (h)	No. of concentrations tested (Range, ppm)	Size (mm)	Temp. (°C)	Conditions of Test	Reference
5	0.98 ppm	36 [480]	5 (1-3)	THE	30	Static	Boltovskoy and Cataldo (2003)
5	1.28 ppm	36 [480]	5 (1-3)	THE	25	Static	Boltovskoy and Cataldo (2003)
5	2.43 ppm	36 [480]	5 (1-3)	THE	20	Static	Boltovskoy and Cataldo (2003)
5	0.88 ppm	48 [480]	5 (1-3)	THE	30	Static	Boltovskoy and Cataldo (2003)
5	1.38 ppm	48 [480]	5 (1-3)	THE	25	Static	Boltovskoy and Cataldo (2003)
5	2.52 ppm	48 [480]	5 (1-3)	THE	20	Static	Boltovskoy and Cataldo (2003)
5	0.90 ppm	720 [0]	5 (0.5-10)	THE	20-22	Static	Boltovskoy and Cataldo (2003)
5	0.71 ppm	264 [0]	5 (0.5-10)	L	20-22	Static	Boltovskoy and Cataldo (2003)
5	34.9 ppm	48 [24-264]	5 (1-30)	A (15-25)	15	Static	Cataldo et al. (2003)
5	1.3 ppm	48 [24-264]	5 (1-30)	A (15-25)	20	Static	Cataldo et al. (2003)
5	1.2 ppm	48 [24-264]	5 (1-30)	A (15-25)	25	Static	Cataldo et al. (2003)
6	%: 41.75	12 [120]	1 (2.3)	A (5-35)	22.5-23.5	FT	Boltovskoy and Cataldo (2003)
6	%: 41.45	24 [120]	1 (2.3)	A (5-35)	22.5-23.5	FT	Boltovskoy and Cataldo (2003)
6	%: 62.15	36 [120]	1 (2.3)	A (5-35)	22.5-23.5	FT	Boltovskoy and Cataldo (2003)

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Agent of control	LC50 or % Mortality	[Post-] Exhibition [exposition] (h)	No. of concentrations tested (Range, ppm)	Size (mm)	Temp. (°C)	Conditions of Test	Reference
?	#: 92.85	48 [120]	1 (2.3)	A (5–35)	22.5–23.5	FT	Boltovskoy and Cataldo (2003)
6	#: 92.05	72 [120]	1 (2.3)	A (5–35)	22.5–23.5	FT	Boltovskoy and Cataldo (2003)
6	#: 63	24 [168]	1 (2.5)	A (6–43)	24–25	FT	Boltovskoy et al. (2005)
6	#: 94	48 [168]	1 (2.5)	A (6–43)	24–25	FT	Boltovskoy et al. (2005)
6	#: 99	72 [168]	1 (2.5)	A (6–43)	24–25	FT	Boltovskoy et al. (2005)
7	7,185 ppm	24 [0]	5 (5–75.5)	L	ND	Static	Darrigran et al. (2001)
8	0.88 ppm	720 [0]	4 (0.5–20)	THE	20–22	Static	Boltovskoy and Cataldo (2003)
8	1.51 ppm	264 [0]	4 (0.5–10)	L	20–22	Static	Boltovskoy and Cataldo (2003)
9	1313.3 ppm	6 [48]	1 (90)	A (15–25)	25	FT	Calazans et al. (2013)
9	270.9 ppm	48 [48]	11 (12–1000)	A (15–25)	25	Static	Calazans et al. (2013)
10	1439.0 ppm	48 [48]	8 (10–10000)	A (15–25)	25	Static	Calazans et al. (2013)
11	8303.1 ppm	6 [48]	4 (90–1000)	A (15–25)	25	FT	Calazans et al. (2013)
11	2536.9 ppm	48 [48]	8 (12–6000)	A (15–25)	25	Static	Calazans et al. (2013)

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Agent of control	LC50 or % Mortality	[Post-] Exhibition [exposition] (h)	No. of concentrations tested (Range, ppm)	Size (mm)	Temp. (°C)	Conditions of Test	Reference
12	45.49 ppm	48 [0]	6 (0.05–500)	A (21–26)	23–27	Static	Montresor et al. (2013) Montresor et al. (2013)
12	13.69 ppm	72 [0]	6 (0.05–500)	A (21–26)	23–27	Static	Montresor et al. (2013)
12	11.10 ppm	96 [0]	6 (0.05–500)	A (21–26)	23–27	Static	Netto (2011)
the 12	%: 99	8760 [0]	1 (1)	THE	18–26	FT	
13	138.54 ppm	24 [0]	ND	L	21.8–23.7	Static	Pereyra et al. (2011)
14	160.21 ppm	24 [0]	ND	L	21.8–23.7	Static	Pereyra et al. (2011)
15	983.27 ppm	168 [0]	ND	A (13)	21.8–23.7	Static	Pereyra et al. (2011)
15	309.92 ppm	168 [0]	ND	A (13)	21.8–23.7	Static	Pereyra et al. (2011)
15	160.1 ppm	168 [0]	ND	A (13)	21.8–23.7	Static	Pereyra et al. (2011)
15	1273.73 ppm	168 [0]	ND	A (19)	21.8–23.7	Static	Pereyra et al. (2011)
15	442.14 ppm	168 [0]	ND	A (19)	21.8–23.7	Static	Pereyra et al. (2011)
15	283.4 ppm	168 [0]	ND	A (19)	21.8–23.7	Static	Pereyra et al. (2011)
15	138.53 ppm	24 [0]	ND	L	21.8–23.7	Static	Pereyra et al. (2011)
16	%: 90	240 [0]	1 (2000)	THE	15–22	Static	Angonesi et al. (2008)
16	%: 92	240 [0]	1 (4000)	THE	15–22	Static	Angonesi et al. (2008)
16	%: 100	240 [0]	1 (6000)	THE	15–22	Static	Angonesi et al. (2008)
16	%: 100	240 [0]	1 (8000)	THE	15–22	Static	Angonesi et al. (2008)

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Agent of control	LC50 or % Mortality	[Post-] Exhibition [exposition] (h)	No. of concentrations tested (Range, ppm)	Size (mm)	Temp. (°C)	Conditions of Test	Reference
16	%: 100	240 [0]	1 (12000)	THE	15–22	Static	Angonesi et al. (2008)
16	8336.7 ppm	48 [48]	8 (1000–20000)	A (15–25)	25	Static	Calazans et al. (2013)
17	376.0 ppm	48 [48]	9 (1–2000)	A (15–25)	25	Static	Calazans et al. (2013)
the 17	%: 86	8760 [0]	1 (1)	THE	18–26	FT	Netto (2011)
18	344.95 ppm	48 [0]	7 (40–800)	A (21–26)	23–27	Static	Montresor et al. (2013)
18	113.14 ppm	72 [0]	7 (40–800)	A (21–26)	23–27	Static	Montresor et al. (2013)
18	88.51 ppm	96 [0]	7 (40–800)	A (21–26)	23–27	Static	Montresor et al. (2013)
the 18	%: 99	8760 [0]	ND	THE	18–26	FT	Netto (2011)
19	%: 2	24 [168]	1 (0.5)	A (6–43)	24–25	FT	Boltovskoy et al. (2005)
19	%: 1	48 [168]	1 (0.5)	A (6–43)	24–25	FT	Boltovskoy et al. (2005)
19	%: 0.2	72 [168]	1 (0.5)	A (6–43)	24–25	FT	Boltovskoy et al. (2005)
19	663.6 ppm	48 [48]	4 (10–1000)	A (15–25)	25	Static	Calazans et al. (2013)
19	%: 100	720–2160 [0]	1 (1)	THE	ND	FT	Cepero (2003)
19	300 ppm: 6 d	144 [0]	9 (0.2–400)	THE	~ 20	FT	Morton et al. (1976)

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Agent of control	LC50 or % Mortality	[Post-] Exhibition [exposition] (h)	No. of concentrations tested (Range, ppm)	Size (mm)	Temp. (°C)	Conditions of Test	Reference
19	400 ppm: 6 d	144 [0]	9 (0.2–400)	THE	~ 20	FT	Morton et al. (1976)
19	200 ppm: 6.5 d	156 [0]	9 (0.2–400)	THE	~ 20	FT	Morton et al. (1976)
19	1 ppm: 15.3 d	367.2 [0]	9 (0.2–400)	THE	~ 20	FT	Morton et al. (1976)
19	0.8 ppm: 23 d	552 [0]	9 (0.2–400)	THE	~ 20	FT	Morton et al. (1976)
20	46.54 ppm	24 [0]	5 (5–80)	A (21–26)	23–27	Static	Montresor et al. (2013)
20	19.84 ppm	48 [0]	5 (5–80)	A (21–26)	23–27	Static	Montresor et al. (2013)
20	14.29 ppm	72 [0]	5 (5–80)	A (21–26)	23–27	Static	Montresor et al. (2013)
20	11.53 ppm	96 [0]	5 (5–80)	A (21–26)	23–27	Static	Montresor et al. (2013)
21	368.2 ppm	48 [48]	6 (10–2000)	A (15–25)	25	Static	Montresor et al. (2013)
22	0.58 ppm	24 [0]	5 (5–80)	A (21–26)	23–27	Static	Montresor et al. (2013)
22	0.35 ppm	48 [0]	5 (5–80)	A (21–26)	23–27	Static	Montresor et al. (2013)
22	0.29 ppm	72 [0]	5 (5–80)	A (21–26)	23–27	Static	Montresor et al. (2013)
22	0.25 ppm	96 [0]	5 (5–80)	A (21–26)	23–27	Static	Montresor et al. (2013)
23	%: 100	720–2160 [0]	1 (1)	THE	ND	FT	Cepero (2003)
24	%: 100	720–2160 [0]	1 (1 ppm Cu ²⁺)	THE	ND	FT	Cepero (2003)
24	%: 100	2160 [0]	3 (0.25–1 ppm Cu ²⁺)	THE	ND	FT	Cepero (2003)

(Conclusion)

MXD toxicity to crustaceans *Macrobrachium amazonicum* was evaluated by Ribeiro and Pelli (2011). The authors found that the product presented acute toxicity at a concentration of 1.93 mg.L⁻¹. In the bioassays, the product generated chemical and biochemical oxygen demand.

7.2.4 Ozone

According to Claudi and Oliveira (2015b), ozone (unregulated, without permission) are in use) provides effective control at concentrations of around 0.15-0.20 mg/L. This technology has a very high cost both in terms of equipment acquisition and generation of the dosages required for population control (DARRIGRAN; DAMBORENEA, 2009). In Brazil, there is little data in the literature on scientific studies testing the effectiveness of ozone, as well as by-products generated by it, although the authors claim that there is no waste production. It is a form of proactive treatment (CLAUDI; OLIVEIRA, 2015b).

7.2.5 Sodium dichlorocyanurate

Through Ibama Normative Instruction No. 18, of October 21, 2015, the product obtained emergency registration, valid for a limited period, only for use in cooling systems of hydroelectric plants, with the objective of creating a microenvironment unsuitable for the fixation of golden mussel larvae, aiming to prevent infestations of the mollusk. The following guidelines of the Normative Instruction must be followed: injection of a sodium dichloroisocyanurate solution, with a concentration of 1.5 mg/L of free chlorine, directly into the water of the cooling system, for two hours per day, with a maximum concentration of 0.01 mg/L of residual chlorine at the effluent outlet; the application of the product must be done using a control system coupled to the cooling system of the generating units of the hydroelectric plant, composed of three distinct units that act in an integrated manner, an automated unit for preparing the solution to be administered; a dosing unit and a unit for reading and automated verification of the active chlorine content, in order to ensure that the indicated dose is correctly maintained during the treatment; a system for monitoring the concentration of residual chlorine and trihalomethanes in the effluent to be released into the water body must be implemented, in addition to other requirements that may be established by the competent environmental agency, in accordance with Conama Resolution No. 467/2015 and Conama Resolution No. 430/2011. It comprises a form of reactive treatment (CLAUDI; OLIVEIRA, 2015B).

Anhydrous sodium dichloroisocyanurate (CAS 2893-78-9) (IBAMA, 2015b) is applied on golden mussel larvae. According to Claudi and Oliveira (2015b), it consists of applications with the slow and constant release of chlorine, in low concentrations, with no formation of trihalomethanes (Table 5).

According to Macedo (2017), this substance has low toxicity, which is why it is widely used in the treatment of swimming pool water. However, there is a lack of studies at different trophic levels to evaluate toxicity in aquatic biota. Macêdo et al. (1999) observed peaks at trace levels in chromatograms of anhydrous sodium dichloroisocyanurate solutions and suggested the need to investigate the possibility of formation of chlorination byproducts, such as chlorinated acetic acid derivatives, haloacetonitriles, hydrated chloral, chloropicrin, chlorophenols and 3-chloro-4-(dichloromethyl)5-hydroxy-2(5H)-furanone (MX), due to their importance to public health.

7.2.6 Other Forms of Chlorine

Chlorine compounds (unregulated, without permission for use), combined with organic compounds, generate trihalomethanes, which are toxic and carcinogenic compounds, while chlorine dioxide does not generate trihalomethanes (DARRIGRAN; DAMBORENEA, 2009). Chlorine dioxide injected continuously into water intake points has been a solution in water treatment plants, demonstrating effectiveness in removing color and odor caused by cyanobacteria, with an 80% reduction in costs for removing scale (CLAUDI; OLIVEIRA, 2015b). According to Darrigran and Damborenea (2009), chlorine gas is toxic, but has proven effectiveness, and the chlorination system is simple and easy to build. According to Claudi and Oliveira (2015b), chlorine gas, liquid sodium hypochlorite, *pellets* of calcium hypochlorite or *pellets* of sodium dichloroisocyanurate (SODI) products are widely used for water treatment plants in South America. Cataldo et al. (2003), Morton et al. (1976) and Fernandes et al. (2012) tested a series of chlorine-based products, the toxicity of which is well known (Table 5). Unlike the lethargic effect caused by copper sulfate on adult golden mussels, the toxicity of chlorine-based products is quickly recognized by the invader, which remains hermetically sealed for some time and then ends up opening the valves due to lack of food, with the action of the controlling agent being determined by the temperature, which influences the resistance of the mollusks (CLAUDI; OLIVEIRA, 2015b).

7.2.7 Veligon [Poly (Dimethyl Diallyl Ammonium Chloride)]

Boltovskoy and Cataldo (2003) tested the effect of this flocculant (unregulated, without permission for use) commonly used in water treatment plants to cause mortality of golden mussels, with a concentration of 50 mg.L⁻¹ being necessary to cause 80% late mortality of golden mussels, after 11 days of application of the product in the system. Blanck et al. (1996) apud Claudi and Oliveira (2015b) reported lower values between 1.5 and 3.0 mg.L⁻¹ to cause mortality of adult zebra mussels. It comprises a form of reactive treatment (CLAUDI; OLIVEIRA, 2015b).

7.2.8 Copper Sulfate

This product is not regulated and is not permitted for use. Upon testing in a bench study, Soares et al. (2009) found that the copper sulfate concentration of 8.48 mg L⁻¹ (equivalent to 1.23 mg L⁻¹ of copper), necessary to cause the mortality of 50% of the golden mussel population, was higher than the standard allowed by the state legislation of Rio Grande do Sul for the release of liquid effluents (0.5 mg L⁻¹ of copper) into surface waters, according to Consema Resolution No. 128/2006, and the standard for class 3 waters (0.013 mg L⁻¹ of copper), according to Conama Resolution No. 357/2005. They are also higher than the concentrations cited by Colares et al. (2002) for the control of the golden mussel (0.5 to 2.0 mg L⁻¹ of copper sulfate). It comprises a form of reactive treatment (CLAUDI; OLIVEIRA, 2015b).

7.2.9 CLAM-TROL CT-2 / Spectrus CT1300

According to Claudi and Oliveira (2015b), they are composed of a family of surfactants cationic (unregulated, without permission for use) widely tested by Boltovskoy and Cataldo (2003), Cataldo et al. (2003) and Boltovskoy et al. (2005), who tested the efficiency of this compound through various designs. Some of these experiments were carried out and the effective concentrations to cause mortality of golden mussels (Table 5) were higher and required more time than the concentrations necessary to cause mortality of dreissenid mussels (CLAUDI; OLIVEIRA, 2015b). It comprises a form of reactive treatment, according to the authors.

7.2.10 H-130 (Didecyl Dimethyl Ammonium Chloride)

According to Claudi and Oliveira (2015b), this liquid compound (unregulated, without permission for use) containing polycanonical alkyl ammonium solution has been registered as a molluscicide for use in industries in North America, but can only be used under strict supervision due to the necessary deactivation procedures prior to disposal. The concentrations tested by Boltovskoy and Cataldo (2003) (Table 5) showed temperature dependence in the efficacy of the product: at 20 °C, 100% mortality after one week of exposure requires doses >10 mg.L⁻¹, while at 25 °C, 2.5 mg.L⁻¹ are sufficient. It comprises a form of reactive treatment (CLAUDI; OLIVEIRA, 2015b).

7.2.11 Bayluscide

Dichloro-2'-nitro-4'-salicylanilide is a molluscicide (unregulated, not permitted for use) widely used in agriculture, tested for the control of mexican

golden finch by Cataldo et al. (2003) (Table 5), who found mortality of up to 90% (0.5-0.8 mg.L⁻¹, 25 °C), a concentration 5 times higher than that required to achieve 100% mortality of *Dreissena* spp. (WALLER et al., 1993 apud CLAUDI; OLIVEIRA, 2015b). The application of this type of product is not recommended for natural environments because it can affect other gastropod molluscs, for which it was originally produced for control purposes, as well as other freshwater bivalves, which are threatened due to habitat changes, invasive species, construction of reservoirs and pollution. Lethal and sublethal effects were observed on native Unionidae bivalves in North America exposed to this molluscicide (NEWTON et al., 2017). It comprises a form of reactive treatment (CLAUDI; OLIVEIRA, 2015b).

7.2.12 Bulab 6002

The product Bulab 6002 (poly [oxyethylene (dimethyliminium) ethylene dichloride tiliminium) ethylene]) (unregulated, no use permit) is a liquid cationic polymerized ammonium compound used to control algae in swimming pools and as a microbiocide to control microorganisms in commercial and industrial water systems (CLAUDI; OLIVEIRA, 2015b). It was tested by Boltovskoy and Cataldo (2003) and Darrigran et al. (2001), demonstrating efficacy in controlling the golden mussel (Table 5). According to Sprecher and Getsinger (2000), this compound presents acute toxicity to shrimp of the order Mysida at concentrations lower (13mg.L⁻¹) than those necessary to cause mortality of the golden mussel (20mg.L⁻¹) and higher than the concentrations to cause mortality of the zebra mussel (2-8mg.L⁻¹). It comprises a form of reactive treatment (CLAUDI; OLIVEIRA, 2015b).

7.2.13 Bioencapsulated

Chemical control agents based on potassium chloride and quaternary ammonia Ternary, microencapsulated by starch, oil and wax binding substance (not regulated, without permission for use), were tested for the control of golden mussels (FERNANDES et al., 2012; CALAZANS et al., 2012 and 2013). Similar methods were developed for the control of Dreissenids. According to Claudi and Oliveira (2015b), this technology releases a smaller quantity of product into the environment, as it is more easily assimilated by the filter feeder, which selects the biocapsules during filtration. However, the registration of permission for use must accompany ecotoxicological tests using native bivalve species, as well as other suspension-feeding macroinvertebrates, which could potentially be affected by this treatment in the aquatic ecosystem. It comprises a form of reactive treatment (CLAUDI; OLIVEIRA, 2015b).

7.2.14 Ammonium Chloride

Montresor et al. (2013) verified the efficiency of syndicated ammonia (TA-N) (not regulated, without permission for use) to cause mortality of adult golden mussels at concentrations ≤ 0.72 mg/L (23 and 27 °C) (Table 5), which would be below the legal standards for the release of this substance, defined in Conama Resolution No. 357/2005 (5.6 mg/L TA-N at pH between 7.5 and 8.0). Thus, ammonium chloride has potential for controlling golden mussels. However, it is necessary to evaluate the toxic effects on biota and obtain registration of the product with Ibama, in accordance with Decree No. 4,074/2002. It comprises a form of reactive treatment (CLAUDI; OLIVEIRA, 2015b).

7.3 Biological Control

There are no field experiences or registered products for the con-biological control of golden mussels (unregulated, without permission for use). The only information refers to bench studies carried out through bioassays, using commercial formulations of microbial agents used for the control of dipterans: Vectobac®, composed of *Bacillus thuringiensis* israelites serotype H-14, Vectobac® aqueous solution (AS), composed of *Bacillus thuringiensis* israelites strain AM65-52 and Vectolex® water-dispersible granules, consisting of the active component *Bacillus sphaericus* Strain 2362. The toxicity of these formulations to adult golden mussels was evaluated by Pereira (2014), who found that Vectobac® AS was the most efficient commercial formulation for controlling golden mussels. However, the lethal concentration of Vectobac® AS for controlling mussels is higher than the effective concentration for killing dipterans. The lowest concentration of Vectobac® AS tested (equivalent to 2.0×10^6 ITU/L against the target dipteran) was 6.7 times higher than the lethal concentration (298 ITU/L) observed by Zequi and Lopes (2007), to kill 95% of a dipteran population, *Culex saltanensis* under laboratory conditions. Furthermore, all concentrations tested revealed an acute toxic effect on the fish *Pimephales pomelas* and the microcrustacean *Ceriodaphnia dubia*, as well as chronic about the algae *Pseudokirchneriella subcaptata*.

7.4 Destination

Conama Resolution No. 467, of July 16, 2015, provides the criteria for the authorization of use of products or agents of physical, chemical or biological processes for the control of organisms or contaminants in surface water bodies. According to the Resolution, one of the requirements for obtaining authorization to use chemical agents is the preparation of a management plan for the solid waste generated, preferably providing for its removal from the surface water body or justification, if this does not occur. Some studies have presented some alternatives for the use of mollusk waste, generated after treatment, as described below.

7.4.1 Soil pH Correction and Fertilization

Through experiments, Barbosa (2009) found that the ground residue of the golden bivalve provided the plants with the equivalent of 24 kg of N ha⁻¹ and 21 kg of P₂O₅ ha⁻¹, which corresponds to relative efficiencies of 31% and 66% of N and P, respectively. Therefore, the agricultural use of bivalve residue was efficient for correcting soil acidity and provided partial supply of nutrients.

7.4.2 Animal Nutrition

Using the golden mussel as raw material, a flour obtained by processing the cooking, grinding and drying process of the bivalve presented average concentrations of 159.173 g/kg for calcium, 73.833 g/kg for protein, 2.52% moisture and pH of 8.13. The sanitary conditions were satisfactory, but the presence of toxic metals should be evaluated, as it may result in restrictions on use in animal nutrition (ALMEIDA et al., 2006).

8. Prevention, Monitoring and Control Actions

The following are prevention, monitoring and control actions carried out by various actors in society, involved with the problem of the invasion of the golden mussel and other species with similar characteristics such as the zebra mussel.

8.1 Other Countries

In the United States, through a national task force (*Aquatic Nuisance Species Task Force*), regional councils worked on developing zebra mussel management plans to establish state and interstate actions, in a decentralized manner, with the involvement of several government agencies. These plans are under federal coordination, although they have strong state influence. The plan developed for the region called *Western US Waters* (WESTERN REGIONAL PANEL ON AQUATIC NUISANCE SPECIES, 2010), focuses on solid preventive actions, based on high priority actions:

- Carry out mandatory inspection and decontamination of infested water immediately after detection;
- Develop effective inspection equipment to monitor vessels, as well as protocols and standards for decontamination;
- Strengthen and enforce laws;

- Develop a standard model and strategy for risk assessment for water bodies;
- Expand inspection capacity and decontamination capabilities to non-infested regions;
- Develop and implement programs to intercept contaminated equipment;
- Early detection of introductions;
- Develop standard field protocols for monitoring and early detection;
- Improve sample analysis methods;
- Coordinate early detection monitoring programs;
- Create and maintain a rapid response fund for eradication and control actions;
- Establish a notification and rapid response database;
- Professional training;
- Containment and control of existing populations;
- Implement reliable and cost-effective control tools;
- Develop control tools in open and closed systems;
- Education.

In Canada, regional management plans have been produced based on efforts similar for the control of dreissenid flies (PROVINCE OF BRITISH COLUMBIA, 2015). These plans include prevention actions, risk analysis, rapid detection, education and information strategies, technical training, scientific research, control, containment and eradication.

8.2 Federal Agencies

The Ministry of the Environment created through Ordinance No. 494, of December 22, In 2003, on an emergency basis, the National Task Force for Mussel Control - golden, resulting in the Emergency Action Plan for the Control of the Golden Mussel, which concludes that: i) the control and containment of the spread of the golden mussel must be permanent tasks; ii) there must be restrictions on waterway traffic activities; iii) inspection actions for small fishing and recreational vessels transported by road must be expanded; iv) greater control over the transport of fauna and flora; and v) advises against the transposition of waters and recommends greater rigor in inspection, in relation to international and coastal navigation.

The following institutions participated in the national task force:

- Ministry of the Environment (MMA) (FTN Coordinator);
- Navy General Staff (EMA);
- Directorate of Ports and Coasts (DPC);
- National Water Agency (ANA);
- National Health Surveillance Agency (Anvisa);
- Brazilian Institute of the Environment and Renewable Natural Resources (Ibama);
- Secretariat of Environment of the Governments of the States of Mato Grosso do Sul and Rio Grande do Sul;
- Municipal Department of Water and Sewage of Porto Alegre (DMAE/RS);
- Brazilian Association of Electric Energy Generating Companies (Abrage);
- Itaipu Binational;
- Furnas Power Plants SA

In 2004, with the purpose of subsidizing studies and filling knowledge gaps, in order to investigate the golden mussel invasion process and its impacts, as an offshoot of the National Task Force, the CNPq organized a research network involving nine institutions, namely: Pontifical Catholic University of Rio Grande do Sul (PUCRS); Maringá State University (UEM); Admiral Paulo Moreira Institute of Marine Studies (IEAPM); Brazilian Agricultural Research Corporation (Embrapa-Pantanal); Federal University of Mato Grosso (UFMT); Federal University of Paraná (UFPR); Pontifical Catholic University of Rio de Janeiro (PUCRJ); Federal University of São Carlos (UFSC); Center for Research and Management of Continental Fisheries Resources (Cepta/Ibama). The coordination was the responsibility of the IEAPM and resulted in an important database on the biology, prevention, monitoring and control of the golden mussel (Process No. 507675/2004), allowing the integration of researchers and the exchange of experiences.

In 2009, the National Biodiversity Commission (Conabio) approved the National Strategy on Invasive Alien Species, according to Conabio Resolution No. 5/2009, which defines guidelines and priority actions for prevention, early detection and rapid response, control, monitoring and eradication of invasive alien species, guiding the different government spheres in dealing with the issue of bioinvasion.

In 2016, the MMA published the book *Invasive Exotic Species of Continental Waters in Brazil*, which recognizes the golden mussel as an invasive alien species. A publication composed of 13 chapters, with data on alien species with invasive potential, already present in the country, their population situations, characteristics, origin, geographic distribution and others, of the taxonomic groups. This publication is available for download at the address: www.mma.gov.br/publicacoes/biodiversidade/category/56-especies-exoticas-invasoras.

Monitoring actions were carried out by the National Water Agency, which implemented a visual inspection project for golden mussels through the national hydrometric network, whose partial data were presented by a water resources specialist from the National Water Agency, Maria Leonor Baptista Esteves. The information is contained in *website* from the MMA, at the following link: mma.gov.br/estruturas/174/_arquivos/174_05122008110517.pdf.

8.3 State Bodies

The Rio Grande do Sul Environment Secretariat (Sema-RS) and the Foundation Environmental Protection Agency (Fepam) held a public hearing to establish a partnership to combat the golden mussel in the Rio dos Sinos in 2007, in partnership with the Rio dos Sinos Hydrographic Basin Committee (Comitesinos), the University of Vale do Rio dos Sinos and the Martim Pescador Institute, seeking to establish control and monitoring measures (<http://www.rs.gov.br/conteudo/151689/sema-e-fepam-formam-parceria-para-combater-me>

xilhao-dourado-n). Fepam participated in a working group to prevent the spread of the golden mussel in the Rio dos Sinos, in conjunction with municipal agencies, discussing prevention and control actions for this basin. In 2003, the government of the state of Paraná launched a campaign to prevent the golden mussel, distributing informative material, in partnership with companies in the electricity sector. Fepam, a member of the National Task Force to combat the golden mussel, carried out monitoring of *L. fortunei* at 58 sampling stations along rivers and dams in the Guaíba hydrographic region, whose partial data are included in Terra et al. (2007), allowing new records in the Taquari River.

The state of Rio Grande do Sul published Ordinance Sema No. 79, of October 31 of 2013, which contains the first list of invasive species in Rio Grande do Sul, which includes species of flora, terrestrial vertebrates, fish, invertebrates and algae, recognizing the golden mussel as an invasive alien species present in the state, classified in category 1, which includes species that are prohibited from being transported, raised, released or translocated, cultivated, propagated (by any form of reproduction), traded, donated or intentionally acquired in any form. The golden mussel was also recognized on the list of invasive species in the state of Paraná (IAP Ordinance No. 74, of April 19, 2007). Subsequently, IAP Ordinance No. 59, of April 15, 2015, again cites the golden mussel among the invasive species present in Paraná, classifying it in the same category adopted by Rio Grande do Sul (category 1). Consema Resolution No. 8 of September 14, 2012, which establishes the list of invasive species in the state of Santa Catarina, does not mention the golden mussel among the invasive species recognized for this state. Consema Resolution No. 30 of 2011 recognizes the golden mussel as an invasive exotic species in the state of São Paulo.

In Santa Catarina, the Licensing Management of Resource Projects

The Water Resources Department chaired a meeting to discuss the control of golden mussels in the state, aiming to integrate other state institutions to raise awareness among the productive sectors involved, create sanitary barriers, and provide guidance for controlling dispersion by fishing and research vessels and equipment. The states of Paraná and Santa Catarina have made progress on the issue of invasive species and have a management action plan. Aware of the issue, the State Program for Invasive Alien Species was implemented in Santa Catarina, through Ordinance No. 116/2016, of the Environmental Foundation. The lines of action include: a) Prevention, early detection; b) Eradication, control, and monitoring: especially aimed at nature conservation units; c) Technical training for prevention, eradication, and control actions; d) Public information: information and education systems; e) Sublegal standards and public policies: regulations, public policies; proposal and review of legal frameworks. Along these lines, actions targeting the golden mussel are being planned.

8.4 Municipal Bodies

In conjunction with Sema/RS, municipal agencies in São Leopoldo (Sema) and Novo Hamburgo (Comusa), as well as state agencies such as Corsan and the Environmental Command of the Military Brigade, met to discuss awareness-raising actions and control of the golden mussel in the Rio dos Sinos Basin. During an environmental monitoring outing in 2014, the Municipal Secretariat for the Environment and Fisheries (Semmap) of the municipality of Imbé (RS) and Ceclimar/UFRGS carried out inspections of the golden mussel in the Tramandaí River.

8.5 River Basin Committees

The Sinos River Basin Management Committee (Comitesinos) works together with state and municipal agencies in the search for strategies to control and monitor the Rio dos Sinos, one of the tributaries of Lake Guaíba, in Rio Grande do Sul. Discussions on the problem of the golden mussel were also established in the Management Committee of the Ibicuí River Basin.

8.6 Reservoirs and Power Generation

In general, contaminated hydroelectric plants carry out forced maintenance three days/year to remove golden mussel incrustations (information obtained through Public Consultation). The costs are high, which has led to the mobilization of the electricity sector to alleviate the problems caused by the golden mussel. The Brazilian Association of Electric Power Generating Companies (Abrage), Itaipu Binacional and Furnas Centrais Elétricas SA acted as member institutions of the National Task Force, which established the first priority actions for the prevention, monitoring and control of the golden mussel in Brazil.

Even without records of golden mussels in its reservoirs, Furnas was a pioneer in prevention activities, as reported by Fillipo et al. (2012), describing actions carried out in the states of São Paulo, Minas Gerais, Goiás, Mato Grosso and Mato Grosso do Sul. In 2012, it collaborated with the dissemination of knowledge, through the book *Invasive Limnic Molluscs in Brazil*, which contains data on species distribution, biological and ecological data, prevention, monitoring and control methods, as well as bases for integrated management.

In partnership with the Government of the state of Paraná, the Institute of Technology for Development (Lactec), in Curitiba, and the Federal University of Paraná (UFPR), in 2003, the companies Companhia Paranaense de Energia (Copel), Itaipu Binacional, Duke Energy – Geração Paranapanema, and Tractebel Energia implemented a campaign to prevent the golden mussel in Paraná (ECOVIAGEM, 2003).

Cesp, aiming to control the golden mussel in the Upper Paraná River, developed extensive awareness-raising work and control techniques.

In 2009, AES Tietê financed and distributed the second edition of the book *Introduction to biology of invasions. The golden mussel in South America: biology, dispersal, impact, prevention and control*, authored by Gustavo Darrigran and Cristina Damborenea, which contains experiences, biological and distribution data and bases for prevention, control and risk analysis. In addition, it distributed informative material about the golden mussel in the state of São Paulo.

Through R&D financed by Cemig, the Species Bioengineering Center was created. Hydroelectric Invading Cities (CBEIH). According to the *website* of the institution (cbeih.org), several research projects were carried out to model the distribution of the golden mussel, assess the risk of dispersion in Brazil, as well as a collaborative database for the immediate updating of new occurrences, launch of alert bulletins of new records and subsidize interinstitutional research.

Cemig carries out the Limnological Monitoring, Water Quality and Control of the Invasive Alien Species Golden Mussel in São Simão, in partnership with CBEIH, which carries out monitoring using the laboratory triad described above. Other companies such as Itaipu Binacional (TAKEDA; FUJITA., 2012), AES Tietê (PEREIRA et al., 2012a) and Furnas (FILLIPO et al., 2012) have carried out monitoring of the golden mussel in their reservoirs, using artificial substrates, examination of structures and quantification of larvae, through optical microscopy. Copel has used a larval detection method based on molecular biology and inspections with robots.

Since 2001, Itaipu has been monitoring larvae and adults in the reservoir and generating unit energy, using mechanical removal, increased flow in pipes, anti-fouling paints, among others (itaipu.gov.br/meioambiente/mexilhao-dourado).

8.7 Water Collection, Treatment and Distribution

The Municipal Department of Water and Sewage of Porto Alegre (DMAE/RS) acted as an institution that is part of the National Task Force, which established the first priority actions for the prevention, monitoring and control of the golden mussel in Brazil.

In the Rio dos Sinos Basin, in Rio Grande do Sul, treatment and distribution agencies water distribution (Semae and Comusa) joined forces to establish control and monitoring strategies in the Rio dos Sinos, in the municipalities of Novo Hamburgo and São Leopoldo. Sanepar, from Foz do Iguaçu, monitored and cleaned the water collection system in Lake Itaipu, using divers and underwater filming in the water collection system. The company performs mechanical cleaning of the set of pipes that collect water from Lake Itaipu

of Itaipu, for the removal of the mollusk (TRIBUNAPR.COM.BR, 2017). Similar processes were carried out by the Municipal Department of Water and Sewage of Porto Alegre (DMAE), in Porto Alegre, and Corsan, in other municipalities of Rio Grande do Sul, resulting in the removal of 300 kg of golden mussels, in a single maintenance stop, according to local news.

According to Bendati et al. (2004), DMAE implemented the following actions for the control of golden mussels: use of 0.5 and 2 mg/L of copper sulfate in raw water piping to remove adult incrustations and control larvae; mechanical removal with a high-pressure jet, with less effectiveness; monitoring of larval forms. According to the authors, consumption of copper sulfate between 2000 and 2003 was 187 tons, reaching a cost of R\$ 615,230.00 (March 2003 values). The cost of divers between September 2001 and December 2002 was around R\$ 22,166.00.

8.8 Aquaculture

For the aquaculture sector, the costs arising from the impact of the golden mussel on the cultivation system are in the order of R\$3,000.00 to R\$4,000.00 per 1,000 kg of dead fish or reduction in the final yield of the fish produced (information obtained through Public Consultation).

Extension actions associated with scientific research were developed in a project funded by Fapesp (No. 14/12553-7), entitled Environmental patterns associated with the development of the golden mussel, *L. fortunei*, in areas with fish farming in net cages, developed in the Canoas II reservoir, Paranapanema River, on the border between the states of São Paulo and Paraná. The project aimed to support actions to prevent, control and mitigate the negative impacts of the invasive species on fish farms. It was coordinated by the São Paulo Agency for Agribusiness Technology (APTA, SAA/SP), with the participation of the Regional Center of Médio Paranapanema, Embrapa-Pantanal; the Center for Environmental Stable Isotopes of Unesp, in Botucatu; the Phycology Research Center, of the Botanical Institute; the Reference Laboratory Unit in Limnology, of the Fisheries Institute and of the Department of Exact Sciences of Unesp in Jaboticabal. They were carried out for two years, establishing relationships between limnological variables of water, sediments, sedimentation rates of suspended material, nutrient availability, phytoplankton community and the development of the golden mussel, which was also investigated in terms of bioindication of fish farming waste, through stable carbon and nitrogen isotopes.

Fepagro Aquaculture and Fishing, in Terra de Areia (RS), carried out from 2014 monitoring of the golden mussel on the banks of Lagoa dos Quadros. Samplers were installed on the banks of the lagoon, in cultivation tanks, experimental units and in the water tank of the Fepagro Research Center.

8.9 Fishing and Navigation

Fillipo et al. (2012) documented pioneering preventive actions carried out by Furnas, together with the fishing industry, distributed informative material, gave interviews on TV and radio, and demonstrated procedures for cleaning boats and fishing nets. These actions were implemented during important fishing festivals held in Cáceres, Mato Grosso, as well as interventions in the states of São Paulo, Minas Gerais, Goiás, Mato Grosso and Mato Grosso do Sul. Presentations and informative leaflets produced and distributed can still be viewed on the company's website (www.furnas.com.br/frmMAAcoesMexilhaoDourado.aspx), which contains instructions on disinfecting fishing gear, oars, anchors, boat hulls, fish tanks and bait on boats. In addition, the company also emphasizes the importance of disposing of water from tanks in other locations. The implementation of control and prevention procedures in fishing gear, water tanks and boat hulls resulted from initiatives by the electricity sector with the support of the scientific community in the states of São Paulo, Minas Gerais, Goiás, Mato Grosso and Mato Grosso do Sul, through environmental education initiatives carried out by Furnas, Cesp and Cemig (FILLIPO et al., 2012).

8.10 Waterways

The same actions implemented by Furnas, described above, contributed greatly to raising awareness and prevention on waterways in Mato Grosso, where fishing boats, among others, are widely used during fishing festivals. These actions were integrated into the education network, involving education secretaries, educators, and members of the Mato Grosso Department of the Environment. The Furnas group worked in partnership with other campaigns carried out by Cesp, which carried out awareness campaigns in riverside communities, using a school boat and a school bus (http://www.cesp.com.br/portalcesp/portal.nsf/V03.02/index_erroacesso?Open-Document).

8.11 Research Institutions

As a result of the actions of the MMA in partnership with CNPQ/CT-Hidro, it was implemented the Research Program for Control of the Golden Mussel in Jurisdictional Waters (MCT/CNPq) was established – Value: R\$ 1,000,000.00, coordinated between 2005 and 2008 by the Almirante Paulo Moreira Institute of Marine Studies.

The project in question involves five subprojects:

Subproject 1: Distribution, population structure, growth and mortality of the golden mussel in Brazil (PUC/RS coordination);

Subproject 2: Chemical and physical control of golden mussels (IEAPM coordination);

Subproject 3: Antifouling paints for mussel control golden (coordination: IEAPM);

Subproject 4: Risk Assessment of reintroduction of the golden mussel rado (coordination: IEAPM);

Subproject 5: Gene identification and study of expression profiles are genetic in *L. fortunei* (coordination: UFRJ).

The Research Program for Control of the Golden Mussel in Jurisdictional Waters was a milestone in research on this invasive species, resulting in numerous dissertations and doctoral theses, articles and book chapters. The institution became a reference on the subject and formed a school of new researchers in partnership with other institutions coordinating subprojects, disseminating knowledge, integrating experiences and discussing the problem of bioinvasion in various events.

The Center for Bioengineering of Invasive Species in Hydroelectric Plants (CBEIH) was created from the Aneel GT-343 R&D, working in monitoring, bioengineering and environmental modeling. Environmental monitoring aims to quickly detect the arrival of an invader, aiming at establishing control and eradication actions, with greater efficiency. The Rapid Detection and Immediate Response Program (DRRI) was developed by this Institute, based on the *Early Detection of Bureau of Reclamation*, a US Government Institute, based on the integration of three detection methods. Sampling is carried out in priority areas indicated by environmental modeling. The results are integrated into a system *online* information, with georeferenced visualization modules and alert systems for immediate decision-making. It operates in a collaborative network with the *Bureau of Reclamation* (an entity linked to the US Department of the Interior), the Department of Mechanical and Aerospace Engineering at the University of California (USA) and the USP Center for Marine Biology (CEBIMa). Among the actions of this center, it is worth highlighting the detection of the first record in the São Francisco River and important modeling studies.

The Laboratory of Studies of *L. fortunei* (LELf) is located in the Center of Hydraulic Research and Water Resources of the Federal University of Minas Gerais (CPH/UFMG). This laboratory was the result of a partnership between CPH/UFMG and the Zoology Department of ICB/UFMG, and was built specifically to work with the golden mussel under biosafety conditions. In 2010, the REALf Research Network (Network for Advanced Studies in *L. fortunei*) was created involving three public universities, the Federal University of Minas Gerais (UFMG), the Federal University of Ouro Preto (UFOP) and the Federal University of Pará (UFPA). The network is coordinated by researchers from the Zoology Department of ICB/UFMG, the CPH/UFMG, the School of Engineering and the Engineering Department of UFOP. In addition to these institutions, REALf has the collaboration of other research centers such as the Instituto de Estudos do Mar Almirante Paulo Moreira (IEAPM), CPqRR/Fiocruz and the *Faculty of Natural Sciences and Museum of the National University of La Plata* (UNLP) – Argentina. The work proposed by REALf is highly interdependent and is developed in a multidisciplinary environment, in which several areas of knowledge interact. This research network has sought and continues to seek advances in knowledge about the golden mussel and in the development of technological solutions, with a focus on reducing energy costs and minimizing the environmental impacts caused by the introduction of the mussel. The network, which aims to integrate researchers from Brazil and other countries, also aims to train specialized and qualified human resources who can help consolidate our initiative.

The Federal University of Rio Grande do Sul (UFRGS), through the Luiz Foundation Englert (FLE), the Ecology Center and the Ceramic Materials Laboratory (Lacer), of the Materials Department (Demat), of the School of Engineering of the Federal University of Rio Grande do Sul (UFRGS), developed a series of projects on species biology, environmental tolerance, monitoring, anti-fouling paints, biological control and physical control, using ultrasound, ultraviolet radiation and ecotoxicology, in partnership with AES Tietê, CEEE, Tractebel, Enerpeixe and Furnas Hydroelectric Power Plants, with a multidisciplinary team of biologists, materials engineers, chemical engineers, among others. The projects resulted in a series of articles, dissertations and theses. The milestone of this research group was the publication of the book *Invasive Limnic Molluscs in Brazil*. The book was financed by Furnas and brought together researchers and project results from the UFRGS research group, as well as researchers from several institutions who worked on the IEAPM project, such as Nupélia, Embrapa, UFMT, other projects carried out at Lactec, actions carried out by the MMA and international contributions from researchers from Argentina and Japan.

In Mato Grosso, it is also worth highlighting the Federal University of Mato Grosso (UFMT), who worked on numerous research projects carried out with IEAPM and UFRGS, among other projects carried out in their own institutions, in which they were involved in prevention, monitoring and ecological studies in the Pantanal, resulting in relevant theses, dissertations and articles, which provided important data on the relationship between the golden mussel

with benthic fauna in wetlands. In Mato Grosso do Sul, Embrapa-Pantanal worked together with other institutions on several physical control projects. In addition, it carried out important scenario forecasts through niche modeling and participated in numerous research projects carried out with IEAPM and UFRGS.

The Institute of Technology for Development (Lactec) has been working since 2003 in conjunction with energy concessionaires from all over Brazil, in the control and monitoring of invasive alien species. The institute carried out constant monitoring work on the reservoirs of five energy companies, to avoid problems such as blockages in the pipes, filters and heat exchangers of the plants. Since 2004, 18 Copel plants have been monitored, in which control methods were studied. Lactec has established agreements with Elejor, Eletronorte, Tractebel and the São Paulo *Duke Energy* and AES Tietê, for specific studies related to the characteristics of each region. The institute also carried out environmental education campaigns, with audiovisual materials distributed in regions that have not yet been affected, and a pioneering study on risk analysis of the spread of the golden mussel in Brazil.

The Federal University of Rio de Janeiro (UFRJ) worked on Subproject 5 (Identification-gene expression and study of gene expression profiles in *L. Fortunei*, of the Research Program for Control of the Golden Mussel in Jurisdictional Waters (MCT/CNPq), coordinated by IEAPM, resulting in several theses in the area of the subproject, contributing to the description of the mitochondrial genome of the golden mussel. Recently, a research project contract was signed with CTG Brasil, to create a genetically modified (GMO) golden mussel, with the purpose of crossing with wild individuals, producing sterile offspring, which would result in population decline. It is worth mentioning that the release of these organisms requires an environmental assessment, allowing verification of the impact on aquatic biodiversity. Furthermore, according to Law No. 7,679, of November 23, 1988, in the breeding of exotic species, it is the responsibility of the aquaculturist to ensure the containment of the specimens in captivity, preventing their access to the drainage waters of the Brazilian river basin. Furthermore, it prohibits the release into the natural environment of genetically modified organisms, whose characterization is in accordance with the terms of specific legislation (Decree No. 8,425, of March 31, 2015).

8.12 NON-GOVERNMENTAL ORGANIZATIONS (NGOs)

The Organized Civil Society entity Ajatar (Friends Jacuí-Taquari), developed see initiatives with the purpose of structuring a laboratory that can operate in its region of coverage with collaborative, scientific and unique research, seeking the development of actions to obtain results that meet the requirements of the Public Civil Action of the Federal Public Ministry of 2007 (information obtained through Public Consultation).

9. Other Applications

Although it is not a species native to South America, several researchers used the golden mussel in environmental assessments as a bioindicator, due to its high filtration capacity, to detect the bioaccumulation of toxic metals, the presence of enterobacteria and to evaluate the mutagenic effects of pollutants.

9.1 Bioaccumulation

Villar et al. (1999) evaluated the concentrations of cadmium (Cd), copper (Cu) and zinc (Zn) in the sedimentary compartment and in tissues of the golden mussel, in the lower stretch of the Paraná River and on the right bank of the Rio de La Plata estuary. The authors detected higher levels of *L. fortunei* in the Paraná River, highlighting the high copper levels (12.05 and 42.0 mg kg⁻¹ of Cu). Samples of the mollusk were analyzed for the presence of toxic metals such as mercury (Hg), cadmium (Cd) and lead (Pb), detected above the legal limit (AGUSTINI; MUCELIN, 2010).

9.1.1 Health Indicator

Salmonella sp., enteric bacteria responsible for serious food poisoning, was detected in a sample of *L. fortunei* collected from a fishing colony in São Miguel do Iguazu (PR), in Lake Itaipu (LANG et al., 2013).

9.1.2 Genotoxicity

Comet and micronucleus tests were standardized using the *L. fortunei* as bioindicator. Exposure to an environmental sample from Lake Guaíba over seven days confirmed the sensitivity of the mussel to water contaminants detected in both tests. The results indicate the golden mussel as a potential bioindicator organism.

The genotoxic potential of surface waters and sediments from the Guaíba Lake Basin was evaluated by Villela et al. (2006) and Silveira et al. (2016), using the comet assay and the frequency of micronuclei in the hemolymph cells of the invasive bivalve *L. fortunei*. Damage was found in the DNA of samples collected in the Itapuã State Park, considered as a reference point, at the mouth of the Arroio Dilúvio and in Lake Guaíba.

10. Final Considerations

Based on the diagnosis presented, in the history of the golden mussel invasion, in Brazil, as well as in all efforts of government agencies, the initiative

private and educational institutions, which resulted in actions, projects and publications on the topic, we can establish considerations and some recommendations.

Brazil began developing actions inspired by international models. national, with the formation of the national task force, involving a series of actors from society and government commissions, which resulted in integration events, the establishment of priority actions and the induction of research projects integrating institutions with *expertise* on the subject and training of human resources.

Scientific advances have resulted in a series of data that have contributed to the understanding of the invasion of the golden mussel in Brazil and can support the prevention and control of this species. The project coordinated by the Instituto de Estudos do Mar Almirante Paulo Moreira (IEAPM), financed with resources from CT-Hidro/CNPQ, resulted in a series of consistent data on biology, ecology, environmental tolerance, chemical control, as well as the genetics of the golden mussel.

Other projects were funded by research and development programs. to, managed by Aneel and with resources from companies in the electrical sector, aiming at research applied to the sector and generating important references such as the book *Invasive Limnic Molluscs in Brazil*. The prediction of occurrence scenarios, based on niche modeling, was carried out considering all Brazilian basins, revealing probabilities of occurrence of the golden mussel in all river systems in Brazil. Only one case of risk analysis, based on vectors, was carried out in the Iguaçu Basin. Studies of this nature are extremely important to estimate probabilities, risks and dangers of the dispersion of this species, based on its dispersion vectors. It is important to emphasize that each basin has its own peculiarities regarding vectors, due to the different human activities and uses of water resources. Knowledge of risks and vectors are key factors for defining prevention actions and for designing monitoring programs in non-invaded river basins, such as the Amazon and Tocantins-Araguaia river basins.

In 2015, the emergency registration of two chemicals (MXD-100 and dichloro-sodium cyanurate), classified as pesticides, was granted for the control of the golden mussel restricted in cooling systems of hydroelectric plants. The use of these products for purposes other than those specified in the registration is prohibited. These were the only chemical products registered, and their use in the natural environment is not authorized.

Prevention actions were carried out by companies in the electricity sector, delaying, in a satisfactory manner, the dispersion of the golden mussel towards the North and Northeast of the country. During a public consultation carried out to evaluate the content of this diagnosis, the need to continue the awareness and consciousness-raising programs

The introduction of new species, especially in the Amazon, Tocantins-Araguaia and São Francisco river basins, was emphasized to prevent the geographic expansion of the golden mussel in Brazil, reaching these basins. Rapid detection of new introductions, so that immediate responses can be made with assertive actions to control population and disperse them into new areas, is extremely necessary for the implementation of effective prevention and control actions. Rapid detection and response programs are key elements in zebra mussel prevention and control plans in the United States, and should be adopted in the Golden Mussel Prevention and Control Plan.

PART II

PLANNING

1. Preparation of the National Plan for the Prevention, Control and Monitoring of the Golden Mussel (*Limnoperna fortunei*) in Brazil

The preparation of the National Plan for the Prevention, Control and Monitoring of the Golden Mussel involved a set of activities and, like the National Plan for the Prevention, Control and Monitoring of Wild Boar (*Your scrofa*), was based on the model and methodology of the National Action Plans for the Conservation of Endangered Species (PAN), according to MMA Ordinance No. 43, of January 31, 2014, and ICMBio Normative Instruction No. 25, of April 12, 2012.

In April 2017, the work plan for the preparation of the Golden Mussel Plan was agreed between the MMA, Ibama and ICMBio, with the definition of the main stages, namely: preparation of a diagnosis on the invasion of the species in the country, holding a public consultation on the diagnosis and a workshop to prepare the plan.

The diagnosis of the golden mussel invasion in Brazil (Part I) was prepared by consultant Daniel Pereira, with supervision from MMA, Ibama and ICMBio.

1.1 Public Consultation

Between October 2nd and 16th, 2017, a Public Consultation was held on the Golden Mussel Plan, through the Ibama website. The consultation aimed to present and gather contributions from society regarding the diagnosis of the invasion of the species. The Consultation received 270 contributions regarding additional records of the species, records of environmental and economic impacts, prevention, control and monitoring actions, in addition to general comments. Researchers, representatives of companies in the electricity sector, civil servants from the federal government, state and municipal governments, transportation companies, sanitation companies, non-governmental organizations, among others, participated in the public consultation. The contributions were analyzed and incorporated into the base document of the Golden Mussel Plan.

Through the Public Consultation, little information was added to the diagnosis. However, weaknesses in the prevention, monitoring and control of the golden mussel in Brazil were highlighted in completed questionnaires, grouped in a summary manner below:

1. Lack of a continuous early detection program in risk areas;
2. Lack of a continuous, integrated and standardized monitoring program to support prevention and control actions;
3. Lack of rapid response protocols aimed at eradicating new invaded areas;
4. There is no control method for use in the natural environment;
5. There is no method of mitigating environmental and economic impacts;
6. Lack of knowledge of the laws on the registration and use of physical, chemical and biological agents to control golden mussels by most technicians and researchers;
7. Lack of training for state and municipal employees;
8. Need for an environmental education program, targeting different users of water resources, with national, regional and local scope, considering the particularities of each region;
9. Vectors are not controlled and monitored;
10. The risks of invasion into new areas are not known;
11. Discontinuity of scientific research lines;
12. Lack of integration between research institutions, the productive sector and river basin committees.

The weaknesses identified during the public consultation served as a basis for the definitions of priority actions during the workshop.

1.2 WORKSHOP

Between November 27th and 30th, 2017, the workshop for the preparation of the National Plan for Prevention, Control and Monitoring of the Golden Mussel (*Limnoperna fortunei*) in Brazil – Plano Mussel-golden, at the National Center for Development and Training of Human Resources of Ibama (Ceduc/Ibama), located at the Ibama Superintendence in the Federal District (Supes-DF). A diverse range of stakeholders participated in the meeting, ensuring the representation of different views on the problem, organization and coordination of actions. In total, 28 people were present, including representatives (Figure 1) of the São Francisco River Basin Committee, the State Secretariat for the Environment of Mato Grosso (Sema), the Caatinga Fauna Management Center (Cemafauna), Companhia Energética de Minas Gerais SA (Cemig), and the Innovation and Technology Center (CIT)

– Senai/Fiemg, São Paulo Energy Company (Cesp), São Francisco Hydroelectric Company (Chesf), Eletrobras, Furnas, Oswaldo Cruz Foundation (Fiocruz), State Institute of Environment and Water Resources (Ibama), Chico Mendes Institute for Biodiversity Conservation (ICMBio), State Institute of Forests (IEF), State Institute of Environment and Water Resources (Inema), Mato Grosso do Sul Environmental Institute (Imasul), Maxclean, Ministry of the Environment (MMA), Federal University of Mato Grosso (UFMT) and Federal University of Rio de Janeiro (UFRJ), totaling 18 institutions, according to the Annex.

During the workshop, the vision for the future was defined, with a time horizon of 25 years; general objective; specific objectives and the planning matrix, containing the actions that make up the Golden Mussel Plan, including coordinator and collaborators, estimated cost, period, location and observations. The Golden Mussel Plan must be implemented within 5 years after its official publication.



Figure 27. Participants in the workshop to develop the National Plan for the Prevention, Control and Monitoring of the Golden Mussel, after the conclusion of activities on November 30, 2017, at the National Center for Development and Training of Human Resources of Ibama, located at the Ibama Superintendence in the Federal District (Supes-DF).

2. Vision of the Future (25 years)

Maintenance of non-invaded basins, without the presence of the golden mussel, with priority given to the Amazon and Tocantins-Araguaia hydrographic regions, and invaded basins with controlled and contained populations.

3. General Objective (5 years)

Prevent the spread of golden mussels into non-invaded areas, as well as contain and control the populations in the invaded areas.

4. Specific Objectives

Six specific objectives were defined for the Golden Mussel Plan, relating to related to the themes of education and awareness, training, scientific research, communication,

prevention and monitoring, control and monitoring and legal framework:

- Specific Objective 1.** Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel;
- Specific Objective 2.** Disseminate information about the golden mussel, forms of prevention and control, as well as the social, environmental and economic impacts of its invasion;
- Specific Objective 3.** Prevent the invasion of golden mussels in new river basins, especially in the Amazon and Tocantins-Araguaia river basins;
- Specific Objective 4.** Implement a standardized inter-institutional monitoring network and a collaborative database;
- Specific Objective 5.** Establish and implement integrated control and monitoring measures for the golden mussel, considering the different types of environments.
- Specific Objective 6.** Evaluate and complement legislation applied to the prevention, control and monitoring of the golden mussel.

5. Planning Matrix

Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel										
No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
1.1	Stimulate research for development to control and containment methods in the environment natural and systems artificial	Methods alternatives identified, scientific article I submit had, notices published		Year 1, Month 1	Continuous	Rogério Sancoughs (UFMT)	0.00	Claudia Callil (UFMT), Otto Mader (Maxclean), Monica Campos (CIT- Senai), Marcela David (Cemig), Maria Cristina Mansur, Marcia Divina (Embrapa Pantanal), Paula Nakayama (Cesp), Thiago Aragão (Chesf), Teofânia Vidigal (UFMG), Mauro Rebelo (UFRJ), Langia Montresor (Fiocruz), Tatiani Chapla (MMA), Ivan Teixeira (Ibama)		National
1.2	Assess the impact of golden mussel in ecosystems aquatic	Articles scientific published, publications officers, notes techniques		Year 1, Month 1	Continuous	Rogério Sancoughs (UFMT)	0.00	Claudia Callil (UFMT), Otto Mader (Maxclean), Monica Campos (CIT- Senai), Marcela David (Cemig), Maria Cristina Mansur, Marcia Divina (Embrapa Pantanal), Carlos Belz (UFPR), Teofânia Vidigal (UFMG), Mauro Rebelo (UFRJ), Karen Larsen (IEAPM), Langia Montresor (Fiocruz)		National

(Continued)

Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
1.3	Update data from occurrence of me-golden squill in the conservation units vation, especially in federal UCs, as well as stimulate carrying out estimates densities population in these areas	Data updated, reports, ar-scientific tigo submitted		Year 1, Month 1	Con-tenuous	Tainah Guimaraes (ICMBio)	0.00	MG		
1.4	Estimate the impact economic of the me-golden squill in the various activities	Reports and publications	Raise awareness the population and the sectors about the impact economic	Year 1, Month 1	Year 2, Month 12	Rogério Sancoughs (UFMT)	100,000.00	Flavio Fernandes (IEAPM), Joéliton Bezerra (Ibama), Andrei Polejak (MCTIC), Elisa Romano (CNI), Marcela David (Cemig), Daercy Ayrosa (Fisheries Institute-SP), Otto Mader (Maxclean), Thiago Aragão (Chesf), Paula Nakayama (Cesp), Carlos Barreira Martinez (UFMG), Alessandro Bearzi (Antaq)		National

(Continued)

Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
1.5	Articulate actions to the creation of notices for research projects related to golden mussel	Notices	Financement for search	Year 1, Month 1	Year 5, Month 12	Marilia Marino (MMA)	10,000.00	Thiago Aragão (Chesf), Daniel Pereira (Lotica), Ivan Teixeira (Ibama), Claudia Callil (UFMT), Otto Mader (Maxclean), Monica Campos (CIT-Senai), Marcela David (Cemig), Maria Cristina Mansur, Marcia Divina (Embrapa Pantanal), Carlos Belz (UFPR), Teofânia Vidigal (UFMG), Paulo Magalhães (UFOP), Flavio Fernandes (IEAPM), Mauro Rebelo (UFRJ), Antonio Valadao (UFOP), Langia Montresor (Fiocruz)	National	National
2.1	Train teachers-res, level technicians medium and higher, fishermen, swimming-worshippers, leaders community and Committee members of Basin to act how to multiply pains in actions of prevention	Educational material captive, courses ministered		Year 1, Month 1	Con-tenuous	Manoel Vieira (CBHSF)	50,000.00	Rodrigo de Fillipo (Concremat), Izabel Chagas (Chesf), Luiza Marilac (Semad-MG), Marcela David (Cemig), Ivan Teixeira (Ibama), Tatiani Chapla (MMA)	National	In special, Hi-Regions druggists Amazonian and Tocantins-Araguaia, and Basin of the Saint Francisco

(Continued)

Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
2.2	Empower managers public (PRF, PMA, Supervised Bodies-res, Oemas, Navy, Map, Seap, between others) for taking of the decision technically based	Educational material captive, courses ministered		Year 1, Month 1	Con- tenuous	Manoel Vieira (CBHSF)	50,000.00	Ivan Teixeira (Ibama), Vitor Domingues (Ibama), Eliese Oliveira (Ibama), Tatiani Chapla (MMA)	National	In special, Hi-Regions Amazonian and Tocantins-Araguaia, and Basin of the Saint Francisco
2.3	Encourage publications in widely circulated magazines and newspapers national and regional with information about the golden mussel	News published		Year 1, Month 1	Con- tenuous	Rogério Sanchugs (UFMT)	0.00	Tainah Guimarães (ICMBio), Manoel Vieira (CBHSF), Ivan Teixeira (Ibama), Marcela David (Cemig)	National	
2.4	Articulate the vehicle-information sharing on the websites and other publications of the institutions participating institutions of this plan	News came-in the sites		Year 1, Month 1	Con- tenuous	Raquel Sabaini (Ibama)	0.00	Tainah Guimarães (ICMBio), Carlos Targino (MMA), Manoel Vieira (CBHSF), Rogério Santos (UFMT), Marcelo Coutinho (Semad-MG), Thiago Aragão (Chesf), Paula Nakayama (Cesp), Marcela David (Cemig)	National	

(Continued)

Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
2.5	Articulate with the communication advisory services communication of organs environmental to be conveyed information sharing on public radio and community	Parts produced, interviews granted		Year 1, Month 1	Continuous	Marilia Marino (MMA)	0.00	Tainah Guimaraes (ICMBio), Juliana Baretta (Ibama)	National	
2.6	Include disclosure of information about the prevention of golden mussels in plans or programs of communication and environmental education of the undertakings licensed instruments of activities with dispersion potential are or introduction of species	Plans with actions related to go to prevention, news broadcasted		Year 1, Month 1	Continuous	Eliese Oliveirara (Ibama)	0.00	Helen Mota (Cemig), Daniel Pereira (Lotica), Ivan Teixeira (Ibama)	National	In special, Hi-Regions Amazonian and Tocantins-Araguaia, and Basin of the Saint Francisco
2.7	Articulate actions to creation of notices for projects of environmental education related to golden mussel	Notices	Recur-sos for education environmental	Year 1, Month 1	Continuous	Emerson Skrabe (Ibama)	0.00	Ivan Teixeira (Ibama), Helen Mota (Cemig)	National	

(Continued)

Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
2.8	Promote the theme of the golden mussel rado in the events technical-scientific	Lectures, workshops, workshops, seminars	Sensitivity-organization of society	Year 1, Month 1	Con- tenuous	Joeliton Bez- zero (Ibama)	50,000.00	Claudia Callil (UFMT), Otto Mader (Maxclean), Monica Campos (CIT-Senai), Marcela David (Cemig), Maria Cristina Mansur, Marcia Divina (Embrapa Pantanal), Paula Nakayama (Cesp), Thiago Aragão (Chesf), Teofânia Vidigal (UFMG), Paulo Magalhães (Ufop), Flávio Fernandez- des (IEAPM), Antonio Valadao (UFOP), Mauro Rebelo (UFRJ), Langia Montresor (Fiocruz), Tatiani Chapla (MMA), Helen Mota (Cemig), Daniel Pereira (Lotica), Ivan Teixeira (Ibama), Tatiani Chapla (MMA), Paulo Bergonci (Cemafauna), Paulo Formagio (Furnas)		National
2.9	Articulate participation of various bodies and sectors of Government, related to the topic, in prevention, monitoring and control of the golden mussel	Agreements of cooperation technique, minutes of the meetings, memories of meeting	Participation active of the several organs and sectors involved	Year 1, Month 1	Con- tenuous	Ugo Vercillo (MMA)	50,000.00	John Paul II (Ibama), Marcela David (Cemig), Eliese Cristina (Ibama), Thiago (Chesf), Vitor Domingues (Ibama), Henrique Anatole (MMA), Daniel Pereira (Lotica)		National 3.1

(Continued)

Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
3.1	Preliminary mapping of the areas relevant to the prevention and invasion of the golden mussel	Maps with relevant areas you have identified to the		Year 1, Month 1	Year 2, Month 1	Helen Mota (Cemig)	30,000.00	Daniel Raíces (ICMBio), Daniel Pereira (Lótica), Paulo Bergonci (Cemafauna), Monica Campos (CIT- Senai), Carlos Belz (UFPR), Otto Mader (Maxclean), Thiago Aragão (Chesf), Flávio Fernandes (IEAPM), Márcia Divina (Embra-		National
3.2	Vector identification of dispersion in river basins of priority areas	Diagnosis with the vetoes-identified res you	Subsidies for the analysis if at risk	Year 1, Month 1	Year 2, Month 6	Paul Bergonci (Cemafauna)	1,000,000.00	Tainah Guimarães (ICMBio), Rogério Santos (UFMT), Claudia Callil (UFMT), Otto Mader (Maxclean), Monica Campos (CIT- Senai), Marcela David (Cemig), Maria Cristina Mansur, Marcia Divine (Embrapa Pantanal), Carlos Belz (UFPR), Teofânia Vidigal (UFMG), Paulo Magalhães (UFOP), Flavio Fernandes (IEA-PM), Antonio Valadao (UFOP), Mauro Rebelo (UFRJ), Langia Montresor (Fiocruz)		Areas Priorities
3.3	Develop protocols for the analysis of risk of dispersal and invasion, considering modeling of spatial distribution of species	Validated protocol given, including of the maps generated	To make available a tool-mint for the analysis of risk	Year 1, Month 1	Year 3, Month 6	Daniel Pereira (Lótica)	1,500,000.00	Carlos Belz (UFPR), Otto Mader (Maxclean), Monica Campos (CIT- Senai), Antonio Valadao (Ufop), Flavio Fernandes (IEA-PM), Marcia Divina (Embrapa Pantanal), Carlos Targino (MMA), Marcela David (Cemig), Helen Mota (Cemig), Claudia Callil (UFMT), Maria Cristina Mansur, Paula Nakayama (Cesp)		National

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Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
3.4	Apply and disseminate analysis protocols risk of dispersion and invasion	Reports, maps and Protocols applied and disclosed	Subsidies for the taking of decision	Year 3, Month 1	Continuous	Joeliton Bezerra (Ibama)	500,000.00	Ivan Teixeira (IBAMA), Monica Campos (CIT-Senai), Marcela David (Cemig), Tainah Guimarães (ICMBio), Rogerio Santos (UFMT), Carlos Belz (UFPR), Zelia Maria Pimentel Nunes (UFPA), Colin Beasley (UFPA), Ezequias Procopio de Brito (UFPA), Rossineide Martins da Rocha (UFPA)		Areas Priorities
3.5	Articulate the elaboration of a protocol with actions for the control of dispersion vectors with institutions located in the areas invaded priorities	Minutes of meetings and Protocol finished	Actions of the protocol for the control of vectors of dispersal implemented of the	Year 1, Month 1	Year 2, Month 1	Fania Campos (Imasul - MS)	100,000.00	Daniel Raíces (ICMBio), Joeliton Bezerra (Ibama), Lea Waksman (Sema-MT), Marcia Divina (Embrapa Pantanal), Ivan Teixeira (Ibama), Rosane Barreto (Inema-BA)		Areas Priorities
3.6	Develop actions joint pre-intervention in the areas relevant invaded	Actions planned, prioritized and I developed of the	None new record in areas priority	Year 2, Month 1	Year 3, Month 1	Fania Campos (Imasul - MS)	100,000.00	Daniel Raíces (ICMBio), Joeliton Bezerra (Ibama), Lea Waksman (Sema-MT), Marcia Divina (Embrapa Pantanal), Ivan Teixeira (Ibama), Rosane Barreto (Inema-BA)		Areas Priorities
3.7	Articulate the elaboration of protocol with prevention actions to the introduction with ins-localized institutions in priority areas not invaded	Minutes of meetings and protocol finished	Actions of the protocol for the control vectors of dispersion implemented of the	Year 1, Month 1	Year 3, Month 1	Fania Campos (Imasul - MS)	100,000.00	Daniel Raíces (ICMBio), Joeliton Bezerra (IBAMA), Lea Waksman (SEMA-MT), Marcia Divina (Embrapa Pantanal), Ivan Teixeira (IBAMA), Rosane Barreto (Inema-BA)		Areas Priorities

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Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
3.8	Develop actions joint prevention in non-areas invaded	Actions planned, prioritized and developed	None new record in areas relevant	Year 2, Month 1	Year 3, Month 1	Fania Campos (Imasul - MS)	500,000.00	Daniel Raíces (ICMBio), Joeliton Bezerra (Ibama), Lea Waksman (Sema-MT), Marcia Divina (Embrapa Pantanal), Ivan Teixeira (Ibama), Rosane Barreto (Inema-BA)		Areas relevant
4.1	Develop a protocol monitoring it standardized for the golden mussel	Protocol developed	Data for-droned and improvement of the information	Year 1, Month 6	Year 2, Month 12	Monica Fields (CIT-Senai)	300,000.00	Paulo Bergonci (Cemafauna), Claudia Callil (UFMT), Otto Mader (Maxclean), Monica Campos (CIT-Senai), Marcela David (Cemig), Maria Cristina Mansur, Marcia Divina (Embrapa Pantanal), Manoel Vieira (CBHSF), Daniel Pereira (Lótica), Tatiani Chapla (MMA), Tainah Guimarães (ICMBio), Ivan Teixeira (Ibama)		National
4.2	Identify the existence-databases on occurrence and biology of the species	Bank of active data		Year 1, Month 1	Year 1, Month 6	Rogério Sancoughs (UFMT)	0.00	Tainah Guimarães (ICMBio), Manoel Vieira (CBHSF), Marcela David (Cemig)		National
4.3	Develop or strengthen-create database collaborative	Bank of data developed	Information available and systematically zadas	Year 2, Month 1	Year 5, Month 12	Joeliton Bezerra (Ibama)	450,000.00	Carlos Targino (MMA), Tainah Guimarães (ICMBio), Tatiani Chapla (MMA), Ivan Teixeira (Ibama), Marília Marini (MMA), Helen Mota (Cemig), Daniel Pereira (Lótica), Paulo Bergonci (Cemafauna), Rogério Santos (UFMT), Vitor Domingues (Ibama), Raquel Sabaini (Ibama)		National

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Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
4.4	Identify act-res-key and compose the network of monitoring	Network implanted		Year 1, Month 1	Year 1, Month 12	Daniel Pereira (Lotica)	5,000.00	Joeliton Bezerra (Ibama), Daniel Raíces (ICMBio), Daniel Pereira (Lótica), Rosane Barreto (Inema-BA), Maria Cristina Mansur, Carlos Belz (UFPR), Otto Mader (Maxclean), Monica Campos (CIT-Senai), Antonio Valadao (UFOP), Flavio Fernandes (IEAPM), Marcia Divina (Embrapa Pantanal), Carlos Targino (MMA), Ivan Teixeira (IBAMA), Lea Waksman (SEMA-MT), Helen Mota (CEMIG), Claudia Callil (UFMT), Colin Beasley (UFPA), Felipe Cardoso (Piagaçu Institute), Daniel Pimpão (IBAMA), Rogerio Santos (UFMT), Manoel Vieira (CBHSF), Daercy Ayrosa (Fishing Institute-SP)		National
4.5	Create a mechanism of warning and early detection for new records of golden mussel	System of alert and detect early action deployed	New invasions detected early	Year 1, Month 1	Year 2, Month 6	Helen Mota (Cemig)	30,000.00	Maria Cristina Mansur, Carlos Targino (MMA), Tainah Guimafrogs (ICMBio), Tatiani Chapla (MMA), Ivan Teixeira (Ibama), Marília Marini (MMA), Helen Mota (Cemig), Daniel Pereira (Ló-ethics), Paulo Bergonci (Cemafauna), Antonio Ostrensky (UFPR), Monica Campos (CIT-Senai)		National

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Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
5.1	Create and implement national registry of golden mussel control initiatives	Register implemented	Centralization and facilitation access to information about the initiatives of control	Year 1, Month 1	Year 5, Month 12	Raquel Sabaini (Ibama)	R\$ 100,000.00	Vitor Domingues (Ibama), Tatiani Chapla (MMA), Ivan Teixeira (Ibama)		National
5.2	Propose guidelines for the monitoring of environmental impacts such methods of control of the golden mussel	Guidelines proposals for organ environmental	Data standardized and improves ment of the information	Year 1, Month 1	Year 2, Month 6	Otto Mader (Maxclean)	R\$ 20,000.00	Marcela David (Cemig), Otto Mader (Maxclean), Vitor Domingues (Ibama), Langia Montresor (Fiocruz), Daniel Pereira (Lótica), Rogerio Santos (UFMT), Ivan Teixeira (Ibama), Eliese Cristina (Ibama), Tatiani Chapla (MMA)		National
5.3	Identify new technologies for the control and monitoring of golden mussel, with priority for scalable technologies ve and automatable	New technologies identified	New technologies prioritized for actions to promote search	Year 1, Month 1	Continuous	Mauro Rebeit (UFRJ)	R\$ 0.00	Marcela David (Cemig), Tatiani Chapla (MMA), Ivan Teixeira (Ibama), Vitor Domingues (Ibama), Daniel Pereira (Lotica), Langia Montresor (Fiocruz),		National
5.4	Develop a manual of guidelines for mussel control-golden, considering at the various impactful activities of the species	Manual elaborated	Improvement of the actions of control	Year 1, Month 1	Year 3, Month 6	Daniel Pereira (Lotica)	R\$ 50,000.00	Vitor Domingues (Ibama), Otto Mader (Maxclean), Marcela David (Cemig), Flavio Costa Fernandes (IEAPM), Ivan Teixeira (Ibama), Eliese Oliveira (Ibama), Langia Montresor (Fiocruz), Daniel Pear Tree (Lotica)		National

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Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
5.5	Include the golden mussel rado as a parameter in monitoring in the licenses of current operations and in the renewal for the activities with potential to be impacted by species, according to the guidelines and protocols those established in action 4.1	Report with data of mussels-golden	Information available and systematically zadas	Year 2, Month 6	Year 3, Month 6	Eliese Oliveirara (Ibama)	R\$ 5,000.00	Ivan Teixeira (Ibama), Carlos Targino (MMA)		National
5.6	Propose the inclusion of golden mussel in the monitoring limnological and water quality current, carried out by public bodies according to the protocol established in action 4.1	Office sent-of, memories of meetings	Inclusion of golden mussel rado in the monitorings current	Year 3, Month 8	Year 4, Month 8	Tatiani Chapla (MMA)	R\$ 5,000.00	Ivan Teixeira (Ibama), Daniel Pereira (Lotica), Paulo Bergonci (Cemafauna)		

(Continued)

Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
5.7	Promote the implementation of technologies environmentally friendly you and economically viable, preferential-scalable minds and automatable, for the control and monitoring of golden mussel	Technologies available-bilized/recommended	Improvements in the processes monitoring systems twisting and control	Year 5, Month 1	Continuous	Mauro Rebeit (UFRJ)	R\$ 50,000.00	Marcela David (Cemig), Otto Mader (Maxclean), Monica Campos (CIT-Senai), Tatiani Chapla (MMA), Ivan Teixeira (Ibama), Eliese Oliveira (Ibama), Vitor Domingues (Ibama), Langia Montresor (Fiocruz), Daniel Pear Tree (Lotica)		National
5.8	Develop protocol quick response to new detections	Protocol elaborated	Agility in taking of decisions	Year 2, Month 6	Year 3, Month 12	Tatiani Chapla (MMA)	R\$ 50,000.00	Marcela Davi (Cemig), Otto Mader (Maxclean), Monica Campos (CIT-Senai), Ivan Teixeira (Ibama), Tainah Guimarães (ICMBio), Vitor Domingues (Ibama), Eliese Oliveira (Ibama), Daniel Pereira (Lótica), Langia Montresor (Fiocruz)		National
5.9	Articulate the implement-rapid response protocol setting for new detections	Protocol implemented	Agility in taking of decisions	Year 4, Month 1	Year 5, Month 12	Tatiani Chapla (MMA)	R\$ 200,000.00	Marcela David (Cemig), Otto Mader (Maxclean), Monica Campos (CIT-Senai), Ivan Teixeira (Ibama), Tatiani Chapla (MMA), Eliese Oliveira (Ibama), Vitor Domingues (Ibama), Langia Montresor (Fiocruz), Daniel Pear Tree (Lotica)	Areas relevant defined in the risk map	National

(Continued)

Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
5.10	Propose guidelines for the destination of mussels from control actions and maintenance	Guidelines proposals	Waste intended adequate-mind	Year 2, Month 6	Year 3, Month 3	Monica Campos (CIT - Senai)	R\$ 20,000.00	Vitor Domingues (Ibama), Marcela David (Cemig), Marcelo Coutinho (Semad-MG), Joeliton Bezerra (Ibama), Otto Mader (Maxclean), Langia Montresor (Fiocruz)		National
6.1	Review the legislation applied to prevention, control and monitoring of golden mussel, identifying gaps, overlays and anti-nomias, and propose alternative necessary rations	Report with the proposal of changes and propositions necessary	Standards suitable proposals	Year 1, Month 1	Year 2, Month 3	Joeliton Bezerra (Ibama)	R\$ 5,000.00	Vitor Domingues (Ibama), Marcelo Coutinho (Semad-MG), Tatiani Chapla (MMA), Raquel Sabaini (Ibama), Eliese Oliveira (Ibama), Flavio da Costa Fernandes (IEA-PM), Lea Waksman (Sema-MT)		National
6.2	Articulate with the Special Secretariat of Fisheries and Aquaculture and the Ministry of Agriculture, Livestock and Supply for proposition of regulations for the trans-control carrying of organisms aquatic continents such alive	Normative proposal	Bigger control of vectors associated with the transport of organisms alive	Year 2, Month 6	Year 3, Month 6	Ivan Teixeira (Ibama)	R\$ 5,000.00	Tatiani Chapla (MMA), Raquel Sabaini (Ibama), Marcela David (Cemig), Otto Mader (Maxclean), Langia Montresor (Fiocruz), Theophania Vidigal (UFMG), Henrique Anatole Ramos (MMA)		National

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Specific Objective 1. Generate scientific data aimed at prevention and control, as well as systematize and make available technical-scientific information on the golden mussel

No.	Action	Product	Results expected	Period		Articulator	Cost estimated (R\$)	Contributors	Location	
				Start	End				Locations	Area of relevance
6.3	Include river ports in the Conama Resolution proposal for risk management of exotic species invaders in ports	Control of the risks in river ports included in normative	Bigger control of vectors associates to the ports	Year 1, Month 1	Year 1, Month 2	Ivan Teixeira (Ibama)	R\$ 5,000.00	Tatiani Chapla (MMA), Eliese Oliveira (Ibama), Otto Mader (Maxclean), Flavio da Costa Fernandes (IEAPM), Alessandro Ramalho (Antaq)		National
6.4	Propose a standard for establish the con-control of the dispersal of exotic species in-sweepers in transport waterways, including the displacement of vessels by land	Standard proposal	Bigger control of vectors associates to transport waterway. Ports controlled	Year 2, Month 6	Year 3, Month 6	Ivan Teixeira (Ibama)	R\$ 20,000.00	Teresa Cristina (Brazilian Navy), Alessandro Ramalho (Antaq), Tatiani Chapla (MMA), Ronildon Miranda of the Santos (PRF)		National
6.5	Set guidelines for the inclusion of the golden mussel theme rado in the processes licensing environmental activities impacted data or with potential for dispersal/introduction	Guidelines defined	Inclusion of guidelines in the processes of licensing-environmental	Year 2, Month 6	Year 4, Month 1	Eliese Oliveirara (Ibama)	R\$ 50,000.00	Marcela David (Cemig), Thiago Aragão (Chesf), Paula Nakayama (Cesp), Tatiani Chapla (MMA), Ivan Teixeira (Ibama), Daniel Pereira (Lotica), Monica Campos (CIT-Senai), Otto Mader (Maxclean), Carlos Belz (UFPR), Fânia Campos (Imasul-MS), Rosane Barreto (Inema-BA), Lea Cintia Waksman (Sema-MT), Paulo Bergonci (Cemafauna), Daniel Pimpan (Ibama)		National

(Conclusion)

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