

## **Analysis of Four Dispersion Vectors in Inland Waters: The Case of the Invading Bivalves in South America**

Author(s): Carlos Eduardo Belz, Gustavo Darrigran, Otto Samuel Mäder Netto, Walter A. Boeger and Paulo Justiniano Ribeiro Junior

Source: Journal of Shellfish Research, 31(3):777-784. 2012.

Published By: National Shellfisheries Association

DOI: <http://dx.doi.org/10.2983/035.031.0322>

URL: <http://www.bioone.org/doi/full/10.2983/035.031.0322>

---

BioOne ([www.bioone.org](http://www.bioone.org)) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/page/terms\\_of\\_use](http://www.bioone.org/page/terms_of_use).

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

## ANALYSIS OF FOUR DISPERSION VECTORS IN INLAND WATERS: THE CASE OF THE INVADING BIVALVES IN SOUTH AMERICA

CARLOS EDUARDO BELZ,<sup>1\*</sup> GUSTAVO DARRIGRAN,<sup>2</sup> OTTO SAMUEL MÄDER NETTO,<sup>1</sup> WALTER A. BOEGER<sup>3,4,5</sup> AND PAULO JUSTINIANO RIBEIRO JUNIOR<sup>3,6</sup>

<sup>1</sup>Universidade Federal do Paraná (CEHPAR), Curitiba, Paraná, Brasil; <sup>2</sup>CONICET División Zoología Invertebrados (GIMIP), Museo de La Plata, FCNyM-UNLP, Paseo del Bosque, 1900 La Plata, Argentina; <sup>3</sup>Universidade Federal do Paraná; <sup>4</sup>Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ), Brazil; <sup>5</sup>Grupo Integrado de Aqüicultura e estudos Ambientais; <sup>6</sup>Laboratório de Estatística e Geoinformação (LEG)—Centro Politécnico da Universidade Federal do Paraná s/n Curitiba, Paraná, Brasil

**ABSTRACT** As a consequence of the current globalization of commerce, natural environments are subject to an unprecedented dynamic transport of organisms because global conditions favor transport, settlement, and dispersal of invading species. These produce widespread impacts such as decreased agricultural and utility production, increased health risks to humans and wildlife, and a significant decrease in native biodiversity. On the assumption that it is better to prevent bioinvasions than to control them, it is of paramount importance to identify and manage the potential dispersal vectors to implement preventive strategies. In our study, we identified 4 potential vectors in southern Brazil (sand transport, attachment to hulls of sports fishing boats, water in sports fishing boats, and live fish) for 2 freshwater invasive bivalves (*Corbicula fluminea* and *Limnoperna fortunei*). For each of these potential vectors, we assess the potential for dispersal by estimating the probability of finding larvae or adults, setting groundwork for further studies on the risks of invasion to which the region is subject.

**KEY WORDS:** invading bivalves, freshwater, vectors, South America

### INTRODUCTION

The value of inland water bodies to humankind is so high that it cannot be overestimated, and any induced changes in the goods and services they provide necessarily have a strong impact on human welfare. For example, Costanza et al. (1997) stated that lakes, rivers, and wetlands currently contribute 20% to the estimated annual global value of the entire biosphere, amounting to an annual figure of US\$33 trillion. Such a huge number may justify the current general concern about the increasing degradation of freshwater systems, associated with the rapid extinction rate of their biodiversity (Gherardi 2007).

Global change and globalization of trade spurred an increase in bioinvasions and their ensuing impact on ecosystems (Darrigran & Damborenea 2011). Many human activities, including agriculture, aquaculture, recreation, transportation, aquarium trade, construction of canals, and other aquatic diversions, promote the dispersal of species beyond their natural barriers (Ruiz et al. 1997, Benson 2000). Consequently, the accelerating invasion of communities by nonnative species is currently a subject of great concern among ecologists, environmentalists, and managers (Orensanz et al. 2002). Invasion of alien species is one of the most important factors endangering global biodiversity because it blurs the uniqueness of regional fauna and flora (IUCN 2000, Xu et al. 2006).

In general, researchers are expected to identify and control pathways of accidental introductions, to promote measures that may prevent unwanted introductions, and to produce protocols for assessment of environmental risk before introduction actually occurs (Gherardi 2007). To address the prevention and control of a bioinvasion, Marco et al. (2002) proposed considering two basic and general aspects in the

study of bioinvasions: one concerning the invasive species and the other concerning the host environment. *Invasiveness* is the ability of a particular species to invade a given habitat, whereas *invasibility* is the susceptibility of a given environment to be invaded (characteristics of a habitat that determine its availability for the establishment and spread of an invasive species).

In such a context, the aim of this work is to detect the most significant vectors of aquatic bioinvasions in the neotropical region. This objective should be met taking into account two premises: (1) identification of locations vulnerable to invasion allows focusing management efforts on the prevention of invasions at those locations and (2) that a transport vector is the conveyor that carries a nonnative propagule to its new location (Mack 2003).

The few available estimates of the number of species moved around the world by vectors suggest that these numbers are huge (Lockwood et al. 2007). In this sense, vectors are the “Achilles’ heel” of bioinvasions (Carlton & Ruiz 2005), allowing a species to conquer a new habitat even if it is far from its native region or current distribution area. Intercepting vectors can reduce the frequency of bioinvasions. According to Carlton and Ruiz (2005), it is important to accumulate knowledge on the diverse human transport mechanisms in different regions that may be potential vectors for invading species. It is therefore necessary to generate databases that indicate dates, history, habitat, and ecological attributes of the detected invasions to assess the range of possible vectors and their rates of success (Ruiz & Carlton 2003). Carlton and Ruiz (2005) suggest that invasions can be reduced drastically by implementing powerful and dynamic vector-managing strategies.

Prime introduction vectors are the unintentional transport by ships (e.g., ballast water, tank sediment, hull fouling) and intentional import of alien species for aquaculture purposes (i.e.,

\*Corresponding author. E-mail: belzoceanos@gmail.com  
DOI: 10.2983/035.031.0322

target species and nontarget species, such as disease agents or parasites) (Gollasch 2007). In addition, Cowie and Robinson (2003) consider the trade of pets and live food. Carlton and Ruiz (2005) propose an appropriate systematization of vector analysis as a promising road toward the prevention of new invasions. The economic and environmental costs of introducing nonindigenous freshwater molluscs are increasing worldwide, as growing numbers of species are transported beyond their native ranges (Keller et al. 2007). Since the late 1960s, at least 2 species of freshwater bivalves have invaded South America: *Corbicula fluminea* (Muller 1774) (Corbiculidae), or Asiatic clam; and *Limnoperna fortunei* (Dunker, 1857) (Mytilidae), or golden mussel. The 2 species share several traits that favor success in their new environment (Darrigran 2002), such as a short life span (2–3 y), rapid growth, rapid sexual maturity, high fecundity, ability to colonize a wide range of habitat types, wide range of physiological tolerance, gregarious behavior, some form of association with human activities (e.g., inhabitants of canals, reservoirs, harbors), high genetic variability, and suspension feeding. In addition, the golden mussel, as seen in other Mytilidae, has a planktonic larval development, which facilitates wide dispersal (Darrigran 2002).

The most efficient way to avoid ecosystem damage by non-native species, such as that caused by *L. fortunei* in South America (Darrigran & Damborenea 2005, Darrigran & Damborenea 2011), is to prevent the introduction of harmful species (Keller et al. 2007). The conceptual model of vector dispersal developed by Carlton and Ruiz (2005) seems to be a promising approach to avoiding new introductions while slowing down dispersal of the golden mussel in the region. Herein we present a regular monitoring program to identify some of the vectors involved in the dispersal of the golden mussel in South America. Implementing the analysis proposed by Carlton and Ruiz (2005) is arduous and difficult in environments such as those invaded by *L. fortunei*. The purpose is to identify and quantify dominant dispersal vectors for adults, juveniles, and larvae of *L. fortunei* and *C. fluminea* in this region (Bolotovskoy et al. 2006, Aguiar 2010). At the same time, we contrast the hypotheses that juveniles and adults can be transported alive during (1) the transportation of sand destined to artificial beaches (sand and gravel are traditionally hauled on roads and highways (Aguirre et al. 2010)), (2) attached to hulls (Ruiz & Carlton 2003), and (3) within live fish. An examination of the lower digestive tracts of fish shows that many of the molluscs are alive (Brown 2007); therefore, survival through gut passage indicates an important role of these vertebrates in the dispersal of freshwater molluscs in freshwater systems (Brown 2007). Fourth, and last, golden mussel larvae may be transported in water in ships and in water used for transport of live fish (Ruiz & Carlton 2003).

## MATERIAL AND METHODS

Our monitoring study accounts for 4 common dispersal vectors for this region: (1) sand transport, (2) attachment to hulls of sports fishing boats, (3) water in sports fishing boats—these potential vectors are the most dominant in the region (Mugetti et al. 2004, Belz 2006)—and (4) live fish.

### Transport by Sand Trucks

Fieldwork for the study of this vector was carried out in the municipality of Guaira (24°17'46.12" S, 54°17'24.74" W), Paraná State, Brazil, after 2 monitoring phases. The 2 invading

bivalves in this area are *C. fluminea* and *L. fortunei* (Zanella & Marenza 2002). There are numerous companies that dredge sand from the reservoir of the huge Itaipú hydroelectric project on the Paraná River. Trucks transporting this sand were sampled randomly between August 12 and 13, 2004. The early field monitoring phase included the following sampling procedures: First, a PVC tube (diameter, 5 cm; length, 60 cm) was attached to a suction system to draw sand from the truckloads. Then, the samples were sieved with a 1-mm<sup>2</sup> mesh and shells were recorded as fragments, bivalve shells, and live specimens. Considering that the specimens undergo mechanical stress—not a physiological one, as in the interior of a digestive tube—those specimens found intact, moist, and with soft parts were considered alive.

Sand was sampled for particle size analysis. To gather information on the origin of the sand, a questionnaire used to determine the place of extraction and the transportation company. Also in the survey were questions about the use and destination of the transported sand, and its volume. The answers allowed us to estimate the trend of the direct impact caused by the vector under study, and the risk of sand transport activity as a vector of molluscs. In addition to learning the origin, destination, and volume of sand, it is also necessary to determine whether there are populations of invading bivalves in the area. Questionnaire data also inform whether the sand is to be used in civil construction or to be put in contact with water bodies, such as in artificial beaches.

We assume the number,  $Y_s$ , of live specimens in each inspection follows a Poisson distribution with parameter  $\lambda = \theta v$ , where  $v$  is the amount of sand and  $\theta$  is the occurrence rate of live specimens per unit of volume of sand. With  $\theta$  estimated from the data, we may estimate the probability of live specimens by computing  $P[Y_s \geq 1]$ . Based on the analysis of the questionnaires, the second phase of monitoring unfolded. It was carried out in the Salto Caxias (25°32'36.54" S, 53°30'24.79" W) Reservoir, Iguazú River, from February 1–3, 2005. The shore was surveyed onboard a ship, and artificial beaches were identified. Random samples of sand were taken from each of them over an area of 1 m<sup>2</sup> to collect specimen shells classified as previously mentioned.

### Transport by Sports Fishing Boats: Water in the Boat and Hull Attachment

Sports fishing boats were monitored from October 22–26, 2005, along the Paraná River in the Club del Municipio de Foz do Iguazú (25°34'34.05" S, 48°35'32.96" W). On removal from the water, boat conditions were recorded and a survey was conducted. To identify adult specimens of the golden mussel, the following processes and data were conducted and recorded, respectively, for each boat: straining of water accumulated in the bottom of the boat (flooding small fishing boats lacking water pumps) with a 64- $\mu$ m mesh for observation under a stereoscopic microscope to identify larvae of the golden mussel (Jimenez Bravo 2004, Ezcurra de Drago et al. 2009), presence of water plants and tree branches attached to or entangled on the vessel (Mansur et al. 2003), and organisms attached to anchors and hulls. The questionnaire included the following questions: For how long have you been fishing today? Do you use live bait? Do you use an anchor while fishing? Do you fish in other regions? If yes, where? Are you aware of macrofouling problems in freshwaters? Do you usually wash your boat after fishing? These questions were intended to build

a profile of the fishery and its risk level in the transport of live larvae and adult mussels, identifying temporal and spatial variables. They were also designed to characterize the transit of fishing boats from other regions.

The probability of finding at least 1 live specimen in each transport mechanism (*Pspecimen*) was estimated as

$$P_{specimen} = P_{exposure} \times P_{transport} \quad [Eq. 1]$$

where *Pexposure* is the proportion of inspected boats exhibiting each of the transport mechanisms (namely, water (either inside the passenger compartment or in the bottom of the hull) or river material (macrophytes) in the boat or attached to the hull, which reflects favorable conditions for transport) and *Ptransport* is the probability of finding at least 1 live specimen (with the number of live specimens given by the Poisson distribution). The probability of finding at least 1 live specimen of *L. fortunei* in fishing boats in the western region of Paraná State is obtained by combining

probabilities for each transport mechanism, considering the union of events rule and assuming independence among them.

Water intake was simulated by pumping at 8 locations along the Paraná River (Fig. 1) to estimate larvae resistance in 100-L tanks used to transport live fish with the same aeration and water conditions as used for commercial fish transport. During the period between consecutive locations, water samples were filtered every 0.0, 0.5, 2.0, and 2.5 hr, and the presence of live larvae was recorded using a stereoscopic microscope.

**Transport by Live Fish**

Fish as vectors of invasion of the golden mussel were assessed by capture from November 18–25, 2005, in 4 regions along the Paraná River (Fig. 1). Estimates were made of the probability of finding live specimens of *L. fortunei* in guts of 5 species—*Pterodoras granulosus* (Valenciennes, 1840) (Dorididae);

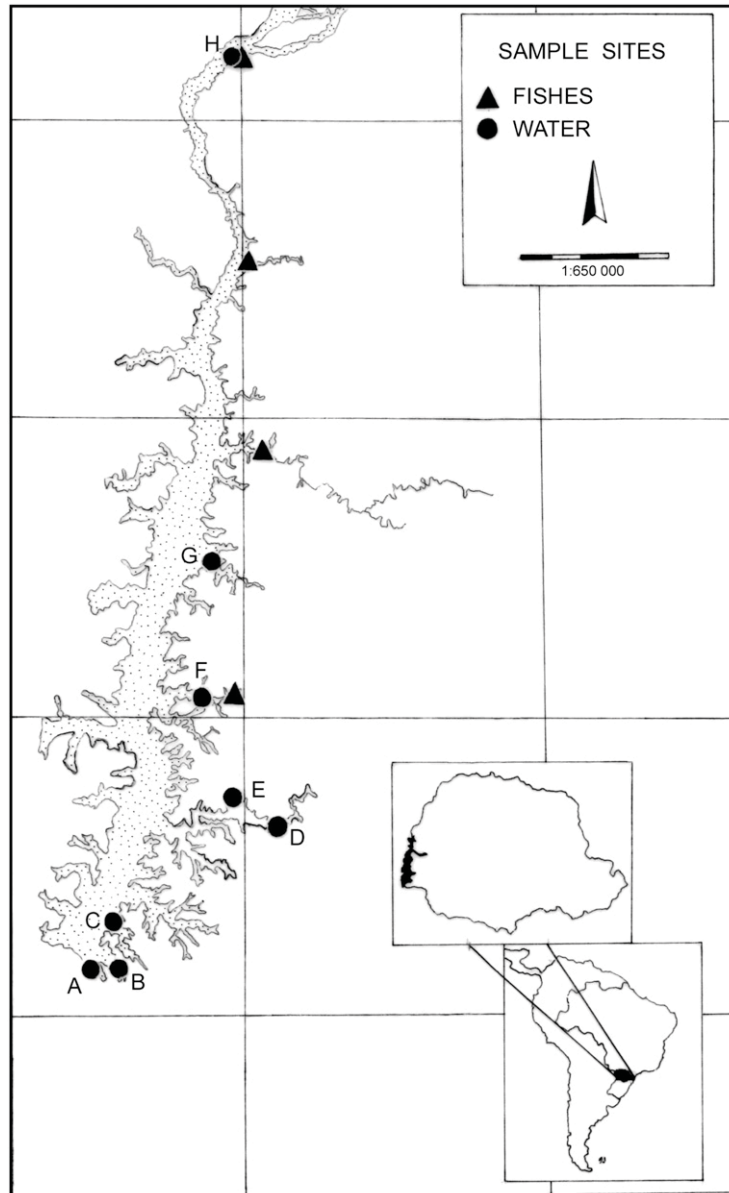


Figure 1. Map showing points of fish collection and water collection for fish transport purposes.



*Satanoperca pappaterra* (Heckel, 1840) (Cichlidae); *Potamotrygon motoro* (Matterer, 1841) (Potamotrygonidae); *Iheringichthys labrosus* (Lutken, 1874) (Pimelodidae); and *Megalancistrus aculeatus* (Perugia, 1891) (Loricariidae). The selection of these species was based on the fact that they are present in the environment (<http://www.fishbase.org>) and feed on *L. fortunei* (Montalto et al. 1999, García & Protogino 2005, García & Montalto 2009). Analysis of the stomach contents of caught fish was performed. After capture, the digestive tubes of each specimen were separated into 4 parts: stomach, upper intestine, middle intestine, and lower intestine.

The content of each part was sieved, separating open and closed shells. Open shells were fixed with 70% ethanol, and the maximum length was measured. Closed shells were placed in aerated water tanks and considered alive if they opened their shells and moved their foot, or closed their shells when stimulated with a pin and then opened their shell again, all within 15 min.

The presence,  $Y_w$ , of whole mussels in the digestive tubes of the selected fish was recorded and follows a Bernoulli distribution with probability  $P_{wholemussels}$  estimated by the proportion of fish with whole mussels. The number,  $Y_L$ , of live mussels, given the presence of whole mussels, is assumed to follow a Poisson distribution, with parameter estimated by the sample average. This allows computing the probability of finding live mussels ( $P_{livemussels}$ ) in the digestive tubes of the fish by

$$P_{livemussels} = P[Y_L \geq 1 | Y_w = 1]P[Y_w = 1]. \quad [\text{Eq. 2}]$$

Similar computations are used to estimate the probability of finding live larvae in the transport water of fish used for pisciculture ( $P_{live larvae}$ ).

Probability calculations throughout the data analysis were based on Bernoulli and Poisson models. The former is used for data recorded as presence/absence whereas the latter is used for modeling counts, such as number of specimens found. The model parameters are estimated by maximum likelihood, with 95% likelihood-based confidence intervals given between brackets expressing the uncertainty in parameter and probability estimates.

## RESULTS

Thirty-two sand trucks belonging to 3 companies were sampled for sand transport of *C. fluminea* and *L. fortunei*. The proportions of trucks sampled from each company were 46.9%, 43.7%, and 9.4%, respectively. The volume sampled ranged from 0.0013–0.0044 m<sup>3</sup>, with a total of 0.1762 m<sup>3</sup> and an average of 0.0055 m<sup>3</sup> per truck. The transported volume ranged from 9–30 m<sup>3</sup>, with an average of 23 m<sup>3</sup>. Table 1 summarizes the particle size analysis. Specimens of *C. fluminea* were found (within the same truck sample) with 15 complete shells and 3 live specimens, measuring an average of 10.5 mm long, and a maximum of 3 live specimens. For *L. fortunei*, there were 5 complete shells and 2 live specimens with an average length of 9 mm, all these values computed for one truck sample (Table 1).

Molluscs were found alive in samples of 4 of the 32 trucks (12.5%). A Poisson model was fitted to account for the different sampled volumes for the number of specimens,  $Y$ . For *L. fortunei*, the estimated mean was 0.0622 specimens/m<sup>3</sup> (range, 0.0103–0.1918 specimens/m<sup>3</sup>). The probability of finding more than 1 specimen per unit of volume (measured in cubic meters) is  $P(Y \geq 1) = 1 - e^{-0.0622} = 0.0603$  (range, 0.0103–0.1745). Analogous results for *C. fluminea* provide an

TABLE 1.

General data from the sand samples collected in the transport trucks and number of molluscs found in the sand samples.

	Company A	Company B	Company C	Total
Sampled trucks ( $n$ )	15	14	3	32
Total sand in sampled trucks (m <sup>3</sup> )	295	271	49	615
Total sampled sand (m <sup>3</sup> )	0.0551	0.1094	0.0117	0.1762
Grains > 2 mm (%)	5.78	43.85	1.58	
Fragments of <i>C. fluminea</i>	2	68	0	70
Complete shells <i>C. fluminea</i>	0	13	2	15
Live specimens <i>C. fluminea</i>	0	3	0	3
Fragments <i>L. fortunei</i>	0	3	0	3
Complete shells <i>L. fortunei</i>	0	5	0	5
Live specimens <i>L. fortunei</i>	0	1	1	2
Total	2	93	3	98

estimated mean of 0.1554 specimens/m<sup>3</sup> (range, 0.0557–0.3340 specimens/m<sup>3</sup>) and  $P(Y \geq 1) = 0.1439$  (range, 0.0542–0.2839). Regardless of the species, the probability of finding a live mollusc in the sand collected from the trucks in the Paraná region is  $P(Y \geq 1) = 0.1955$  (range, 0.0892–0.3434).

The effectiveness of trucks carrying sand as a vector of *L. fortunei* is low, because 93.7% of the sand is transported to construction sites. Only 6.3% of the remaining sand is used for development and maintenance of artificial beaches (Fig. 2). Monitoring the beaches (12 beaches in the Salto Caxias, Iguagu River Reservoir) where these trucks were flushed, we found *C. fluminea* densities of 50/m<sup>2</sup> and *L. fortunei* densities of 7/m<sup>2</sup>. All specimens found dead, but still in a good state of preservation.

Thirty-four sports fishing boats were sampled for transport of *L. fortunei*, and the results are summarized in Table 2. A total of 109.7 L was collected in the bait well found in 23 of the boats, with the number of larvae ranging from 0–8, with an average of 1.8 per boat and 0.56/L. For the water collected from the bottom of the boats, a total of 24.5 L was recorded in 10 of the boats with only 3 larvae in total (i.e., 1 boat with 1 larvae and another boat with 2 larvae). Branches and debris were found in the interior of 17 of the boats, with the number of specimens of *L. fortunei* ranging from 0–23 (average, 2.56 specimens per boat

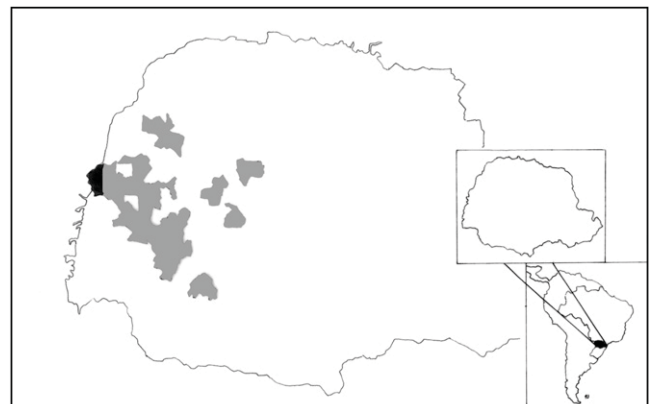


Figure 2. Map showing areas identified as destination of sand collected in the Guaira region. Black, origin locality; gray, destination locality.

TABLE 2.

Material collected associated with transport mechanisms of larvae, juveniles and adults of *L. fortunei* in a total of 34 sports fishing boats in the Paraná River, and their probability of occurrence.

Transport Mechanism	Life State	Collection Material	Boats with Collection Material	Pexposure (%)	Ptransport (%)	Pexposure × Ptransport (%)
Nursery	Larva	Water	23	68	44.04	29.79
Bottom of boat	Larva	Water	10	29	7.77	2.28
Macrophytes in boat interior	Juveniles and adults	Macrophytes	0	0	0	0
Branches and residues in boat interior	Juveniles and adults	Branches and residues	17	50	99.4	49.7
Exterior of hull	Juveniles and adults	Biofilm	0	0	0	0
Anchors	Juveniles and adults	Biofilm	0	0	0	0

and 5.12 specimens per boat with material). No specimens were found on the hull or anchor.

Table 2 also shows *Pexposure* and *Ptransport* computed for each transport mechanism. For the latter, the considered median volume of water was 5 L and 2.5 L for bait well water and water in the hull, respectively. The product of the probabilities reflects how effective transport can be when considering boats with specimens in transport mechanisms. Assuming independence between the mechanisms, the probability of finding adult specimens or larvae of *L. fortunei* in the fishing boats within the study region is estimated as  $P = 0.54$ .

When answering the questionnaire, 20% of the 34 sports fishing boat owners stated they fished in more than 1 region. After learning about the presence of invading species, 50% of them considered they could have been responsible for the dispersal.

Mussels were found in the digestive system in 3 of the 5 analyzed fish species, but live mussels were found in *P. granulosus* only (Table 3). Most live *L. fortunei* were found in the stomach, but some were collected from the final portion of the digestive tract, having survived the enzymatically rigorous conditions of this environment. This suggests that some fish species are important dispersal vectors for invading *L. fortunei*, and also suggests that fish could be a natural vector in its native range, too.

Whole mussels were found in the digestive tube of 29 of 46 inspected fish, yielding an estimated probability of  $P_{wholemussels} = 0.6304$ . The probability of live mussels is 0.00204.  $P_{livemussels} = 0.00129$  is the estimated probability of finding live mussels in the fish intestine. Estimated mussel average length and associated SE are  $6.4 \pm 3.1$  mm for *M. aculeatus* and  $14.8 \pm 6.6$  mm for *P. granulosus*, with a statistical difference ( $P < 0.05$ ).

Live larvae of *L. fortunei* were found in all 4 evaluations in 7 of the 8 sites where water was pumped to mimic the usage for live fish transport (Fig. 3, Table 4). Two major trends are evident: (1) a decrease in the number of live larvae along the 4 evaluation times and (2) a large variation in larval density in the samples considered (0–6,326 specimens/m<sup>3</sup>). The proportion of samples with larvae shows the probability  $P_{livelarvae} = 0.875$ . To assess the probability of fish transport as a vector, we can combine 2 mechanisms—mussels in fish intestines (*Plivemussels*) and larvae in transport water (*Plivelarvae*)—and assuming independence, in collection points along the Paraná River,  $P_{livemussels}$  or  $P_{livelarvae} = 0.8752$ .

DISCUSSION

Management of vectors of biological invasions entails interruption of the transfer process (Ruiz & Carlton 2003). Because vectors are the most vulnerable and manageable part of the invasion sequence, their management and prevention leads to a quantifiable reduction in species invasions (Carlton & Ruiz 2005). Vector efficiency is related directly to the economic activity of each region and is crucial for identification of the most important vector for each species. Reported results identify the dispersal vectors of freshwater invading bivalves in a subtropical climate area of the neotropical region: boats (e.g., sports boats, recreational boats, and fishing boats), sand transport, and pisciculture. The finding that the golden mussel (*L. fortunei*) survived passage through the digestive tract of certain fish species suggests an additional dispersal vector.

TABLE 3.

Total numbers of *L. fortunei* (whole and live) found in the digestive tube of the captured fish species.

Species	Fish (n)	Fish with Live Golden Mussels		Mussels in the 3 Parts of the Intestine (n)		Live Mussels in the Final (Lower) Part of the Intestine (n)	
		Golden Mussels in Their Intestine (n)	Mussels in the Stomach (n)	Intestine (n)	Live Mussels in the Stomach (n)	Intestine (n)	
<i>Pterodoras granulosus</i>	29	3	339	2,198	70	4	
<i>Megalancistrus aculeatus</i>	8	0	610	1,913	0	0	
<i>Satanoperca papaterra</i>	3	0	0	0	0	0	
<i>Potamotrygon motoro</i>	2	0	0	0	0	0	
<i>Iheringichthys labrosus</i>	4	0	0	1	0	0	
Total	46	3	949	4,112	70	4	

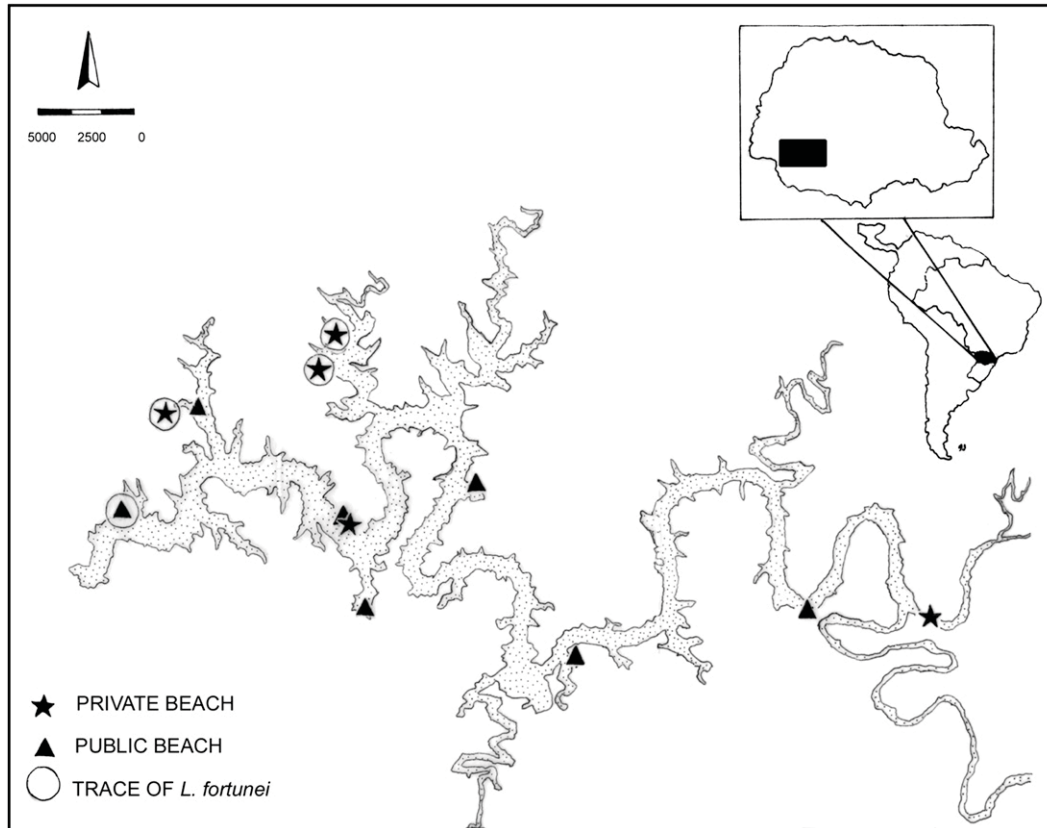


Figure 3. Map showing artificial sand beaches visited around the Salto Caxias Reservoir, Iguaçu River, Brazil.

Despite the fact that only 6.3% of sand is used for infilling of artificial beaches in freshwater reservoirs in Paraná State, transport by sand was another important vector for the study area. Two aspects should be taken into account. The first is the resistance of bivalves to sand loading and unloading methods. Valves found well preserved in the sand had a maximum length of 9 mm for the golden mussel (*L. fortunei*) and 10.5 mm for the Asian clam (*C. fluminea*). Gonad maturity can be reached in specimens 5 mm long in the golden mussel (Darrigran et al. 2007), and in specimens with a shell length of 12–15 mm in the case of *C. fluminea* (Jimenez Bravo 2004). It should be mentioned that, during sand transport, some specimens were found alive. The sand

extraction process is not stressful enough to cause mortality, which enhances sand transport as a vector. The second aspect is the resistance to desiccation shown by transported specimens (Darrigran et al. 2004), considering air exposure tolerance experiments. The mortality rate for *L. fortunei* was 100% of specimens after 7 days of exposure. In a similar experiment, McMahon (1979) recorded a survival rate of up to 26.8 days for *C. fluminea* at 20°C and high relative humidity. During the current study, we recorded live specimens of both species in trucks that contained sand harvested 6 hr before sampling. No live specimens were found in trucks with sand loaded 1 mo earlier. This result suggests that an effective way of eliminating the sand transport vector is to allow a period of at least 30 days to elapse before the sand is used in artificial beaches.

A proportion of 95% of the inspected vessels sailing in the Paraná River use water storage compartments. Although water stored in the compartments is removed when the vessels are away from port areas, live mussels were still found after they were cleaned. This fact renders boats a vector, with a potential risk for other regions eventually reached by the vessels, adding to the fact that the chance of finding adult specimens or larvae of *L. fortunei* in fishing boats within the study region was estimated to be 74%. The potential risk is reinforced by the fact that 20% of fishing boat owners stated that they fished in other regions and their boats were transported by water or over land. After a briefing on the bioinvasion by freshwater bivalves, 50% of those interviewed recognized themselves as potential dispersal vectors of the golden mussel. This highlights the importance of developing public awareness programs on bioinvasions.

TABLE 4.

Number of live larvae per cubic millimeters of *L. fortunei* found in the fish transport tank at different times.

Collection Point	Time 0	Time 0.5	Time 2	Time 2.5
A	54	180	18	18
B	16,326	13,410	8,946	8,100
C	6,93	1,854	3,978	3,600
D	54	108	72	72
E	18	18	0	18
F	450	144	72	54
G	72	54	36	36
H	0	0	0	0

Time 0, collection time; Time 0.5, 30 min after collection; Time 2, 2 hr after collection; Time 2.5, 2.5 hr after collection.

Cleaning of the water storage compartments of the vessels by boats owners reduces the chances of transport by this vector. However, branches, macrophytes, and wood with attached adults of *L. fortunei* are an effective vector, as the species shows a tolerance to desiccation of approximately 170 h (Darrigran et al. 2001). Adults are also found in tubes of refrigeration systems in boats (Darrigran 2002). Johnson and Carlton (1996) also consider vessels as vectors in the case of the zebra mussel *Dreissena polymorpha* (Pallas, 1771) in the northern hemisphere. Recreation, sports, and fishing boats have been associated frequently with the dispersal of invading species from connected water bodies (Griffith et al. 1991, Carlton 1993, Buchan & Padilla 1999). Johnson et al. (2001) noted the remarkable importance of this vector in the dispersal of *D. polymorpha* in North America. Water deposited in the bottom of the boat floor (similar to that seen in *L. fortunei* in the current study) as well as water in the engine refrigeration system (Darrigran 2002) are the main transport agents for larvae and adults. Last, there is a close relation between the aquatic macrophytes present in vessels and the presence of attached adult specimens of *D. polymorpha*, which is in agreement with that observed for *L. fortunei* in the current study. Thus, river boats appear to be the main dispersal vector.

Surviving fish gut passage has not been recognized as an important dispersal mechanism for freshwater molluscs, even though Haynes et al. (1985) documented the phenomenon for a single fish–snail pair. Live bivalves are passing entirely through the gut of some fishes (Haynes et al. 1985, Brown 2007, Loo et al. 2007), suggesting that this could be a natural vector in native bivalve environments. This is a highly significant result in understanding the high rate of upstream dispersal of *L. fortunei* (240 km/y) in the neotropical region (Darrigran 2002). Hence, its spread within catchments may be assisted by fish, especially upstream (Haynes et al. 1985).

#### ACKNOWLEDGMENTS

This work was funded by Companhia Paranaense de Energia, Copel, Programa de Pesquisa e Desenvolvimento da Agência Nacional de Energia Elétrica—Aneel, Brazil; Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura da Universidade Estadual de Maringá, Brazil; and CONICET (PIP 1017-Argentina). We thank M. Lagreca (Comisión de Investigaciones Científicas, Bs.As.-CIC) for technical support.

#### LITERATURE CITED

- Aguilar, J. C. 2010. Stretching the border: smuggling practices and the control of illegality in South America. New Voices series 6. Global Consortium on Security Transformation Santiago. Facultad Latinoamericana de Ciencias Sociales (FLACSO). 25 pp.
- Aguirre, A. B., W. T. Hennes & A. Marks. 2010. Innovative logistics for the transportation of sand and gravel in Brazil. *IJLSM* 7:490–506.
- Belz, C. E. 2006. Análise de risco de bioinvasão por *Limnoperna fortunei* (Dunker, 1857): um modelo para a Bacia do Rio Iguaçu, Paraná. Tese do grau de Doutor em Ciências. Universidade Federal do Paraná. 102 pp.
- Benson, A. J. 2000. Documenting over a century of aquatic introductions in the United States. In: R. Claudi & J. H. Leach, editors. Nonindigenous freshwater organisms: vectors, biology, and impacts. Boca Raton, FL: Lewis Publishers. pp. 1–32.
- Boltovskoy, D., N. Correa, D. Cataldo & F. Sylvester. 2006. Dispersion and ecological impact of the invasive freshwater bivalve *Limnoperna fortunei* in the Río de la Plata watershed and beyond. *Biol. Invasions* 8:947–963.
- Brown, R. J. 2007. Freshwater mollusks survive fish gut passage. *Arctic* 60:124–128.
- Buchan, L. A. J. & D. K. Padilla. 1999. Estimating the probability of long-distance overland dispersal of invading aquatic species. *Ecol. Appl.* 9:245–265.
- Carlton, J. T. 1993. Dispersal mechanisms of the zebra mussel *Dreissena polymorpha*. In: D. W. Nalepa & C. R. C. Schloesser, editors. Zebra mussels: biology, impact and control. Ann Arbor, MI. Lewis (CRC Press). pp. 677–697.
- Carlton, J. T. & G. M. Ruiz. 2005. Vector science and integrated vector management in bioinvasion ecology: conceptual frameworks. In: H. A. Mooney, R. N. Mack, J. A. McNeely, L. Neville, P. Schei & J. K. Waage, editors. Invasive alien species: a new synthesis. Washington, DC: Island Press. pp. 36–58.
- Costanza, R., R. D'Arge, R. De Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'Neill, J. Paruelo, R. G. Raskin, P. Sutton & M. van den Belt. 1997. The value of the world's ecosystem services and natural capital. *Nature* 387:253–260.
- Cowie, R. H. & D. G. Robinson. 2003. Pathways of introduction of nonindigenous land and freshwater snails and slugs. In: G. Ruizand & J. T. Carlton, editors. Invasive species: vectors and management strategies. Washington, DC: Island Press. pp. 93–122.
- Darrigran, G. 2002. Potential impact of filter-feeding invaders on temperate inland freshwater environments. *Biol. Invasions* 4:145–156.
- Darrigran, G. & C. Damborenea. 2005. A South American bioinvasion case history: *Limnoperna fortunei* (Dunker, 1857), the golden mussel. *Am. Malacol. Bull.* 20:105–112.
- Darrigran, G. & C. Damborenea. 2011. Ecosystem engineering impacts of *Limnoperna fortunei* in South America. *Zoolog. Sci.* 28:1–7.
- Darrigran, G., C. Damborenea & N. Greco. 2007. An evaluation pattern for antimacrofouling procedures: *Limnoperna fortunei* larvae study in a hydroelectric power plant in South America. *Ambio* 36:575–579.
- Darrigran, G., M. Maroñas & D. Colautti. 2004. Air exposure as a control mechanism for the “golden mussel” *Limnoperna fortunei* (Dunker, 1857) (Bivalvia, Mytilidae). *J. Freshw. Ecol.* 19:461–464.
- Ezcurra de Drago, I. L. Montalto & O. B. Oliveros. 2009. Desenvolvimento e ecologia larval do *Limnoperna fortunei*. In: G. Darrigran & C. Damborenea, editors. Introdução a biologia das invasões: o Mexilhão Dourado na América do Sul: biologia, dispersão, impacto, prevenção e controle. São Carlos, SP: Cubo Editora. pp. 77–87.
- García, M. & L. Montalto. 2009. Os peixes predadores do *Limnoperna fortunei* nos ambientes colonizados. In: G. Darrigran & C. Damborenea, editors. Introdução a biologia das invasões: o Mexilhão Dourado na América do Sul: biologia, dispersão, impacto, prevenção e controle. São Carlos, SP: Cubo Editora. pp. 111–126.
- García, M. & L. C. Protogino. 2005. Invasive freshwater molluscs are consumed by native fishes in South America. *J. Appl. Ichthyol.* 21:34–38.
- Gherardi, F. 2007. Biological invasions in inland waters: an overview. In: F. Gherardi, editor. Biological invaders in inland waters: profiles, distribution, and threats. Dordrecht: Springer. pp. 3–25.
- Gollasch, S. 2007. Marine vs. freshwater invaders: is shipping the key vector for species introductions to Europe? In: F. Gherardi, editor. Biological invaders in inland waters: profiles, distribution, and threats. Dordrecht: Springer. pp. 339–345.
- Griffith, A., D. W. Schloesser, J. H. Leach & W. P. Kovalak. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. *Can. J. Fish. Aquat. Sci.* 48:1381–1388.



- Haynes, A., B. J. R. Taylor & M. E. Varley. 1985. The influence of the mobility of *Potamopyrgus jenkinsi* (Smith, E.A.) (Prosobranchia: Hydrobiidae) on its spread. *Arch. Hydrobiol.* 103:497–508.
- IUCN. 2000. Guidelines for the prevention of biodiversity loss caused by alien invasive species. Presented at the 51<sup>st</sup> meeting of the IUCN Council, Gland, Switzerland.
- Jimenez Bravo, S. 2004. Biología reproductora, desarrollo larvario y dinámica poblacional de *Corbicula fluminea* (Muller, 1774) (Bivalvia: Corbiculidae) en el Río Miño (Pontevedra, España). PhD diss., Universidad Complutense de Madrid. 203 pp.
- Johnson, L. E. & J. T. Carlton. 1996. Post-establishment spread in large-scale invasions: dispersal mechanisms of the zebra mussel *Dreissena polymorpha*. *Ecology* 77:1686–1690.
- Johnson, L. E., D. I. Ricciardi & J. T. Carlton. 2001. Overland dispersal of aquatic invasive species: a risk assessment of transient recreational boating. *Ecol. Appl.* 11:1789–1799.
- Keller, R. P., J. M. Drake & D. M. Lodge. 2007. Fecundity as a basis for risk assessment of nonindigenous freshwater molluscs. *Conserv. Biol.* 21:191–200.
- Lockwood, J. L., M. Hoopes & M. P. Marchetti. 2007. Invasion ecology. Singapore: Blackwell Publishing. 304 pp.
- Loo, S. E., R. P. Keller & B. Leung. 2007. Freshwater invasions: using historical data to analyse spread. *Divers. Distrib.* 13:23–32.
- Mack, R. N. 2003. Global plant dispersal, naturalization, and invasion: pathway, modes and circumstances. In: G. M. Ruiz & J. T. Carlton, editors. *Invasive species: vectors and management strategies*. Washington, DC: Island Press. pp. 3–30.
- Mansur, M. C., C. Pinheiro dos Santos, G. Darrigran, I. Heydrich, C. T. Callil & F. P. Cardoso. 2003. Primeiros dados quali-quantitativos de Mexilhão-Dourado, *Limnoperna fortunei* (Dunker), no Delta do Jacuí, no Lago Guaíba e na Laguna dos Patos, Rio Grande do Sul, Brasil e alguns aspectos de sua invasão no novo ambiente. *Rev. Brasil. Zool.* 20:75–84.
- Marco, D., S. Páez & S. Cannas. 2002. Species invasiveness in biological invasion: a modelling approach. *Biol. Invasions* 4:193–205.
- McMahon, R. F. 1979. Tolerance of aerial exposure in the Asiatic freshwater clam, *Corbicula fluminea* (Muller). In: Proceedings of the First International Corbicula Symposium, J. C. Britton, Ed. Texas Christian University Research Foundation (Ft. Worth), pp. 227–241.
- Montalto, L., O. B. Oliveros, I. Ezcurra de Drago & L. D. Demonte. 1999. Peces del Río Paraná medio predadores de una especie invasora: *Limnoperna fortunei* (Bivalvia, Mytilidae). *Rev. FABICIB* 3:85–103.
- Mugetti, A. C., A. T. Calcagno, C. Brieva, M. S. Giangiobbe, A. Pagani & S. Gonzalez. 2004. Aquatic habitat modifications in La Plata River basin, Patagonia and associated marine areas. *Ambio* 33:78–87.
- Orensanz, J., E. Schwindt, G. Pastorino, A. Bortulus, G. Casas, G. Darrigran, R. Elias, J. J. Lopez Gappa, S. Obenet, M. Pascual, P. Penchaszadeh, M. L. Piriz, F. Sacarbino, E. D. Spivak & E. A. Vallarino. 2002. No longer a pristine confine of the World Ocean: a survey of exotic marine species in the southwestern Atlantic. *Biol. Invasions* 4:115–143.
- Ruiz, G. M. & J. T. Carlton. 2003. Invasion vectors: a conceptual framework for management. In: G. M. Ruiz & J. T. Carlton, editors. *Invasive species: vectors and management strategies*. Washington, DC: Island Press. pp. 459–504.
- Ruiz, G. M., J. T. Carlton, E. D. Grosholz & A. H. Hines. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extends, and consequences. *Am. Zool.* 37:621–632.
- Xu, H., S. Qiang, Z. Han, J. Guo, Z. Huang, H. Sun, S. He, H. Ding, H. Wu & F. Wan. 2006. The status and causes of alien species invasion in China. *Biodivers. Conserv.* 15:2893–2904.
- Zanella, O. & L. D. Marenda. 2002. Ocorrência de *Limnoperna fortunei* na central hidrelétrica de Itaipú. In: V Congresso Latinoamericano de Malacologia. T. Kawano, Ed. São Paulo: Instituto Butantan/ Instituto de Biociências, USP. pp. 41.