

Research article**Forecasting the expansion of the invasive golden mussel *Limnoperna fortunei* in Brazilian and North American rivers based on its occurrence in the Paraguay River and Pantanal wetland of Brazil**Márcia D. Oliveira^{1*}, Stephen K. Hamilton² and Claudia M. Jacobi³¹Embrapa Pantanal, Rua 21 de Setembro, 1880, CP 109, CEP 79320 900, Corumbá, MS, Brazil²W.K. Kellogg Biological Station and Dept. of Zoology, Michigan State University, 3700 E. Gull Lake Drive, Hickory Corners, MI 49060-9516 USA³Federal University of Minas Gerais, Av. Antonio Carlos, 6627, CEP 31270-901, Belo Horizonte, MG, BrazilE-mail: mmarcia@cpap.embrapa.br (MDO), hamilton@kbs.msu.edu (SKH), jacobi@icb.ufmg.br (CMJ)

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Abstract

The bivalve *Limnoperna fortunei* (Dunker, 1857), also called golden mussel, is native to Asia but becoming dispersed around the world. The golden mussel resembles the invasive dreissenid bivalves in many respects, and although much less studied it evidently has broader environmental tolerances. The golden mussel was introduced into the La Plata River estuary (South America) and quickly expanded upstream to the north, into the tropical Paraguay River reaching a large floodplain area in Brazil known as the Pantanal wetland. The golden mussel tolerates environmental conditions in the Pantanal that would be inhospitable for most bivalves, but mussel mortality has been observed during the most extreme oxygen depletion events. Based on knowledge about the limiting factors for the golden mussel in the Pantanal wetland, its potential distribution was predicted for the remainder of the Paraguay River basin where the species is not present, as well as in other river systems throughout Brazil. Forecasts of potential distribution in Brazilian river systems were based on physicochemical limitations for shell calcification, and specifically on lower thresholds of dissolved calcium concentrations and the calcium carbonate (calcite) index of saturation, which may be a better indicator of calcification potential in low-calcium waters than calcium concentration alone. In addition to examining spatial patterns in calcium and calcification potential, these and other limnological and climate variables were used in ecological niche modeling using GARP and Maxent algorithms. Forecasts of potential distributions in three major North American river systems (Mississippi, Colorado, and Rio Grande) were based mainly on water temperature because calcium availability and calcification evidently would not be limiting to golden mussel establishment in those waters. Due to the greater tolerance of the golden mussel to conditions known to limit other bivalves, as well as its greater ability for shell calcification in low-calcium water, the golden mussel could potentially become broadly distributed throughout Brazil. According to its thermal tolerance *L. fortunei* could become established in the Mississippi, Colorado and Rio Grande drainage systems, although the northern Mississippi River system including the Missouri River may be too cool in the winter to support the golden mussel.

Key words: biological invasions, ecological niche modeling, invasive species**Introduction**

Three invasive freshwater mollusks, apparently dispersed via transoceanic shipping, have become well established in the Western Hemisphere, causing numerous environmental problems, as well as economic costs. The zebra mussel *Dreissena polymorpha* (Pallas 1771), family Dreissenidae, has spread widely across North American fresh waters since its introduction into the Laurentian Great Lakes in the 1980's, resulting in costly impacts due to biofouling and inducing ecological changes throughout aquatic food webs (Schloesser and Nalepa 1994; Caraco et al. 1997; O'Neill 1997;

Strayer et al. 1999; Bykova et al. 2006). The spread of *D. polymorpha* has been followed to some extent by the closely related quagga mussel *D. rostriformis bugensis*. The golden mussel *Limnoperna fortunei* (Dunker 1857), family Mytilidae, became established in southern South America in 1990 and is producing similar economic and ecological impacts (Darrigran and Escurra de Drago 2000; Darrigran 2002; Boltovskoy et al. 2006; Oliveira et al. 2006).

In order to estimate the potential dispersion and eventual geographic distribution of invasive species, many studies have considered their means of transport to a new location, as well as establishment and integration of the invader into

the new environment (Moyle and Light 1996; Shea and Cheson 2002). The rapid expansion of the zebra mussel in North America has resulted from propagule spread via water craft (Bossenbroek et al. 2007; Colautti et al. 2006) and generally favorable physical and chemical characteristics that facilitate growth and reproduction (Ramcharan et al. 1992). In the cases mentioned above other distinctive characteristics not found in native species, including byssal threads for attachment and a planktonic larval phase (veliger) for dispersal, have contributed to success in colonization of new environments (Johnson and Carlton 1996; Johnson and Padilla 1996; Bobeldyk et al. 2005).

Considering that the means of introduction are present in most environments, the expansion of these invasive species can be forecasted on the basis of characteristics of the receptor environment in relation to their ecological niches (Peterson and Vieglais 2001). Two examples where ecological niche modeling has been applied are the prediction of potential distribution of *D. polymorpha* in North America (Drake and Bossenbroek 2004; Bossenbroek et al. 2007) and the expansion of *L. fortunei* globally (Kluza and McNyset 2005). These models have shown good results over large spatial scales using climatological, geological and topographic spatial data.

Global-scale analyses have shown that *L. fortunei* could likely colonize most waters of South America, including the Amazon, Orinoco and Magdalena river systems, where this species has not yet been recorded (Boltovskoy et al. 2006). It has been proposed that *L. fortunei* may also be able to colonize warmer waters in the southern United States where *D. polymorpha* does not occur, on the basis of both species' thermal tolerances and the chemical characteristics of southern US waters (Ricciardi 1998; Kluza and McNyset 2005; Boltovskoy et al. 2006; Karatayev et al. 2007). These studies, however, only provide information at very coarse geographic scales and do not account for limnological variation within broad climatic regions.

For finer-scale forecasts of distributions in specific water bodies, limnological variables such as calcium concentration and pH have been used to predict the distribution and density of the invasive mussel *D. polymorpha* in North America (Ramcharan et al. 1992; Neary and Leach 1992; Mellina and Rasmussen 1994). Low concentrations of calcium and low pH are

suspected to reduce population density or restrict the establishment of bivalves since in most mollusks shell formation (calcification) requires a threshold level of available calcium and a pH range that does not inhibit calcification (Mackie and Flippance 1983; Ramcharan et al. 1992; Checa et al. 2007). Temperature can be important as well because *L. fortunei* has a relatively high tolerance to warmer water temperatures compared to *D. polymorpha* (Morton 1982; Ricciardi 1998; Boltovskoy et al. 2006; Karatayev et al. 2007).

The aim of this study was to forecast the geographic expansion (i.e., risk of establishment) of *L. fortunei* in Brazilian and North American river systems based on their physical and chemical variables. These variables are not commonly used in regional-scale models. The advantage of modeling the potential distribution is that the possibility of an invasion can be assessed before the actual introduction of a species occurs (Peterson and Vieglais 2001). Results from this study can be used to establish priority areas for monitoring, management, and biodiversity conservation as *L. fortunei* expands its range in the Americas.

Methods

Data compilation and modeling

The potential distribution of *L. fortunei* in the Brazilian river systems was predicted through an exploratory analysis based on limnological data integrated into two niche models that have previously been used to predict alien species invasions: GARP (Genetic Algorithm for Rule-set Production), <http://openmodeller.sf.net> and Maxent (Maximum Entropy Method), <http://www.cs.princeton.edu/~chapiere/maxent>, version 3.0.4 beta. Detailed descriptions of the GARP algorithm are presented by Stockwell and Noble (1992), Peterson and Cohoon (1999), Stockwell and Peters (1999), and Anderson et al. (2003). Maxent modeling is described by Phillips et al. (2004 and 2006). Both GARP and Maxent models are based on presence records.

Brazilian river systems modeling followed procedures outlined in Oliveira (2009). To test the accuracy of the GARP and Maxent models we used the receiver operating characteristic (ROC) analysis, which characterizes the performance of a model at all possible thresholds by a single number, the area under the curve (AUC) (Phillips et al. 2004 and 2006). The AUC can be

interpreted as the probability that a model set correctly predicts presence in a randomly selected grid cell. The higher the AUC, the more sensitive and specific the model set, ranging from 0.5 (random accuracy) to a maximum value of 1.0 (perfect discrimination). Only models that presented an AUC above 0.75 were considered in this study. To assess the soundness of the predictions we also used a threshold value in Maxent (Phillips et al. 2006). Values below these thresholds were considered to have conditions of low suitability for *L. fortunei* occurrence.

Limnological variables used in the modeling included water temperature, dissolved oxygen, conductance, pH, calcium concentration, and the calcite saturation index (SI_{calcite}). SI_{calcite} reflects the thermodynamic propensity for calcification, calculated from water temperature, pH, conductance, total alkalinity (Gran 1952), and dissolved calcium, following APHA (2005). The SI_{calcite} is usually interpreted on a \log_{10} scale where zero indicates thermodynamic equilibrium with respect to CaCO_3 , a positive index indicates supersaturation, and a negative index indicates undersaturation. We did not use total suspended solids (TSS) and sestonic chlorophyll *a* in the models, but we used these variables to compare quantity and quality of food among different aquatic systems.

The tolerances of *L. fortunei* are not known for all of these variables. We considered the minimum pH, calcium and SI_{calcite} requirements for *L. fortunei* development to be 6.0, 1.0 mg l^{-1} and -4.0, respectively, based on field observations previously made in the Paraguay River basin (Oliveira 2009). Above these limits, we considered that waters could potentially support *L. fortunei* invasion, and below these limits that the risk of invasion was low.

At low dissolved Ca concentrations, the SI_{calcite} is likely to provide a better indicator of the suitability of a particular water body for shell calcification. The relationship between calcium concentration and SI_{calcite} is specific to a particular water body, reflecting its hydrogeochemical composition and aquatic ecosystem metabolism (a control on dissolved CO_2 concentrations). Typically there is a curvilinear relationship between dissolved Ca concentrations and SI_{calcite} in natural waters, but at low concentrations of dissolved Ca (i.e., $<5 \text{ mg/L}$), the relationship is not strong and SI_{calcite} can vary over more than 2 orders of magnitude for a given Ca concentration, as shown in an example from the Amazon River (Figure 1).

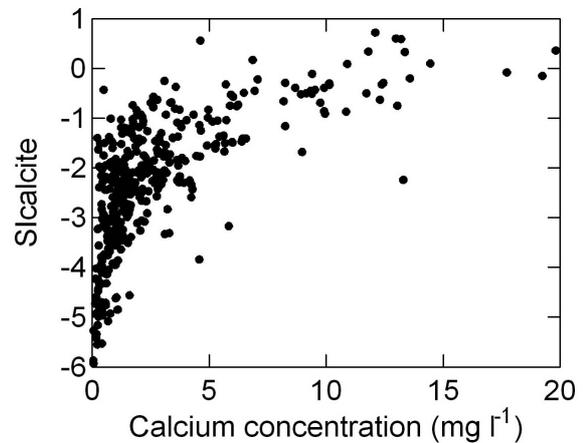


Figure 1. Relationship between the calcite saturation index (SI_{calcite}) and dissolved Ca concentrations in rivers throughout the Amazon River basin (based on a nationwide synoptic survey; data downloaded from <http://www.brasildasaguas.com.br> and SI_{calcite} calculated as described in the text).

Limnological data for the Brazilian river systems are from the ‘Brasil das Águas’ project (<http://www.brasildasaguas.com.br>) except for the Paraguay River basin, where data are from Embrapa Pantanal. We used 786 sampling sites located along six systems: Amazon, Paraná/Paraguay, São Francisco, Tocantins-Araguaia, North Atlantic coastal drainages, and South Atlantic coastal drainages. Except for the Paraguay system, each site was represented by a one-time synoptic survey conducted between 2003 and 2004 for the aforementioned program. We only considered environments with salinity below 12.0 PSU, thereby remaining within the tolerance of *L. fortunei* (Ricciardi 1998). We used 24 records of occurrence in the Paraguay basin for the Brazilian river system models, plus six sites in the Paraná River.

To forecast the expansion of *L. fortunei* in North America we considered three major river systems: the Mississippi (with its Missouri and Ohio River tributaries), the Rio Grande, and the Colorado. Limnological data were taken from the US Geological Survey’s NASQAN program (description available at <http://pubs.usgs.gov/wri/wri014255/>). We analyzed data for 23 sites consisting of means from monthly sampling between 1995 and 2000. We considered the same set of limnological variables used for the Brazilian rivers, including water temperature,

dissolved oxygen, conductance, pH, dissolved calcium concentration, total suspended solids (TSS), and the calcite saturation index (SI_{calcite}), calculated as described above.

The North American rivers had dissolved Ca concentrations, pH, SI_{calcite} , and dissolved oxygen concentrations that were well above the estimated minimum requirements for *L. fortunei* establishment based on our field observations in the Paraguay River and Pantanal wetland, and thus the limnological data were not used to model the potential distribution of *L. fortunei* in North American rivers. Given their favorable water quality but seasonally low temperatures, we focused on the mean minimum water temperature (from December to February, 1995 to 2000) as the main physicochemical limitation to *L. fortunei* establishment in these North American rivers.

Experimental determination of thermal tolerance

Previous estimates of the low-temperature tolerance of *L. fortunei* have been based on observations on the occurrence of *L. fortunei* in waters subject to seasonally cool temperatures. In the Paraguay River basin, occasional cold fronts can bring sudden temperature drops during the winter, but protracted periods with water temperatures below $\sim 20^{\circ}\text{C}$ are uncommon. Therefore we performed laboratory tests where adult *L. fortunei* from the Paraguay River were acclimated at 26°C (the temperature of Paraguay River water when mussels were collected) and then gradually exposed to lowering temperatures, eventually reaching 0, 5, and $10^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. A treatment at 26°C was maintained during the experiment period as a control. There were 9 replicates for 0°C and 5 replicates for each other tested temperature. Each replicate consisted of 20 mussels with shell length between 8-13 mm in a 600-mL beaker. The treatments were maintained in a thermostatically controlled incubator. Additionally, we used an external digital thermometer to check the water temperature inside the beaker.

The period of acclimation for 26°C was around 20 days, followed by daily decreases of 1°C until attaining the target temperatures. The experiment was monitored during 30 days after the water reached the target temperature. No additional food or aeration was provided; rather, the water in the beakers was replaced with water from the Paraguay River every day for the control and every week for treatments with

temperatures $\leq 10^{\circ}\text{C}$, in which the mussels were relatively inactive. The characteristics of the Paraguay River water collected periodically for the experiment were: temperature $26\text{-}27^{\circ}\text{C}$, dissolved oxygen above 6.0 mg l^{-1} , pH between 6.0 and 7.0, and conductance between 70 and $90\ \mu\text{S cm}^{-1}$. Dissolved oxygen and pH were monitored once a day and temperature was monitored every 3 hours. During the incubation dissolved oxygen was $\geq 1.0\text{ mg l}^{-1}$, and the pH and conductance were in the same range as in the Paraguay River. Mussels were checked for survival daily by examining open shells and looking for movement. Mussels that were considered dead were transferred to fresh water at 26°C and checked for movement for about 12 hours. The results were expressed in survival rate per day.

Results

Limnological characterization of Brazilian river systems

Conductance among Brazilian aquatic systems ranged widely from 0.0 to more than $7,000\ \mu\text{S cm}^{-1}$ (Figure 2A). The waters of highest ionic strength were in the Atlantic coastal drainages (North and South), although some streams in these regions can have waters with very low conductance. The Amazon River system also had a large variation in conductance, and included the most ionically dilute and acidic waters. The Paraguay, Paraná, Tocantins-Araguaia, and São Francisco systems had conductances around $100\text{-}200\ \mu\text{S cm}^{-1}$. Conductance was around $400\ \mu\text{S cm}^{-1}$ in the Uruguay River system. Values of pH between 7.0 and 8.0 were most common, but the pH was sometimes lower than 6.0 in the Amazon and Paraguay systems (Figure 2B).

Mean water temperature in the Brazilian rivers was between $25\text{ and }29^{\circ}\text{C}$. Mean dissolved oxygen was higher, ranging from 3.1 and 8.0 mg l^{-1} . Chlorophyll *a* means ranged from 0.0 to $10.0\ \mu\text{g l}^{-1}$ in the Paraguay and São Francisco systems, 10 to $100\ \mu\text{g l}^{-1}$ in the Amazon, Tocantins-Araguaia and North Atlantic systems, and higher than $100\ \mu\text{g l}^{-1}$ in the Paraná and South Atlantic systems.

Calcium concentrations ranged from as low as 0.0 up to 20 mg l^{-1} in most rivers, and exceeded 100 mg l^{-1} in some Atlantic coastal rivers (Figure 2C). Waters from the Amazon, Tocantins-Araguaia, and Paraguay rivers can have Ca concentrations less than 1.0 mg l^{-1} . Most of the

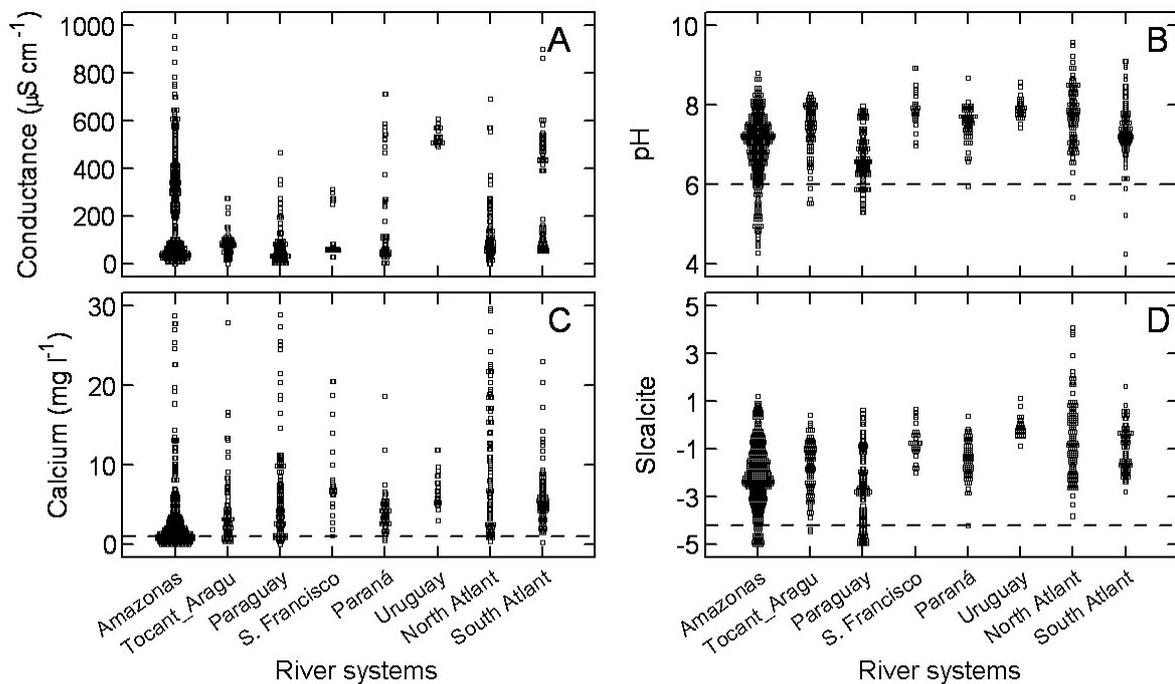


Figure 2. Ranges of conductance (A), pH (B), calcium concentration (C), and SI_{calcite} (D) in the Brazilian river systems. Limnological data from synoptic surveys were provided by “Brasil das Águas Project” except data for the Paraguay River system, which came from Embrapa Pantanal (see text for details). The dashed lines depict approximate lower thresholds for *L. fortunei* establishment based on field observations in the Paraguay River basin (Oliveira 2009).

Brazilian river systems had less extreme undersaturation with respect to CaCO_3 than the Paraguay River, except for some sites in the Amazon system, and most were above the minimum estimated SI_{calcite} required for the establishment of *L. fortunei* based on our field observations in the Paraguay River (SI_{calcite} threshold ~ -4.0) (Figure 2D).

Forecasting the expansion of *L. fortunei* in Brazilian river systems

The same procedures used to forecast the potential distribution of *L. fortunei* in the upper Paraguay basin (Oliveira 2009) were employed to forecast the occurrence of *L. fortunei* in other major Brazilian river systems. The main physico-chemical controls of the potential distribution of *L. fortunei* appear to be the dissolved calcium concentration and SI_{calcite} . These variables indicated that most waters in Brazil have medium to high risk of establishment (Figures 3A and 3B). Included in the regions with high risk are the estuaries of the Amazon and Tocantins rivers,

some coastal drainages to the Atlantic (North and South) such as the Mearim River estuary (State of Maranhão) and the Jequitinhonha, Mucuri, Doce, and Paraíba do Sul rivers. Medium to low probabilities were found for some tributaries of the Amazon River, such as the Negro, and portions of the Tapajós and Xingu rivers, as well as the Araguaia River.

Results from both the GARP and Maxent models using SI_{calcite} (Figures 4A and 5A) showed fewer regions with high risk of *L. fortunei* establishment in the Brazilian rivers than did empirical forecasting using calcium concentration and SI_{calcite} , although most environments presented medium to high risk of *L. fortunei* establishment. The Maxent combined variables model indicated far fewer sites with favorable conditions for *L. fortunei* establishment than the GARP model. GARP and Maxent models using SI_{calcite} predicted medium risk of *L. fortunei* establishment in the Uruguay River and Guaíba Lake where the species is already present.

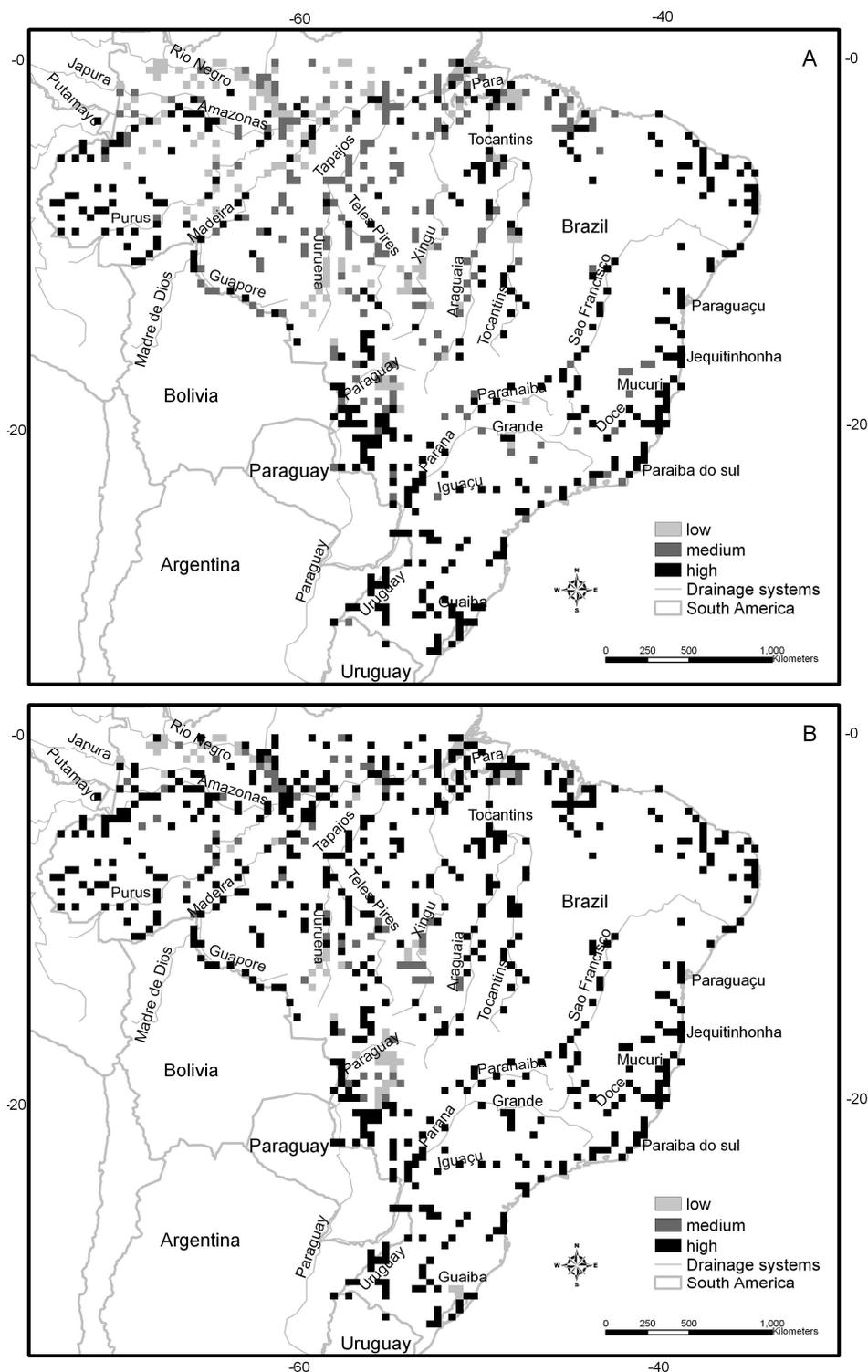


Figure 3. Potential distribution of *L. fortunei* in the Brazilian river systems based on lower thresholds of calcium concentration (A) and $SI_{calcite}$ (B). We considered low risk of *L. fortunei* occurrence in rivers where the mean calcium concentration was between 0.0 and 1.0 mg l⁻¹ and $SI_{calcite}$ was < -4.0, medium risk between calcium concentrations of 1.0 and 3.0 mg l⁻¹ and $SI_{calcite}$ between -4.0 and -3.0, and high risk where calcium concentration was >3.0 mg l⁻¹ and $SI_{calcite}$ was > -3.0.

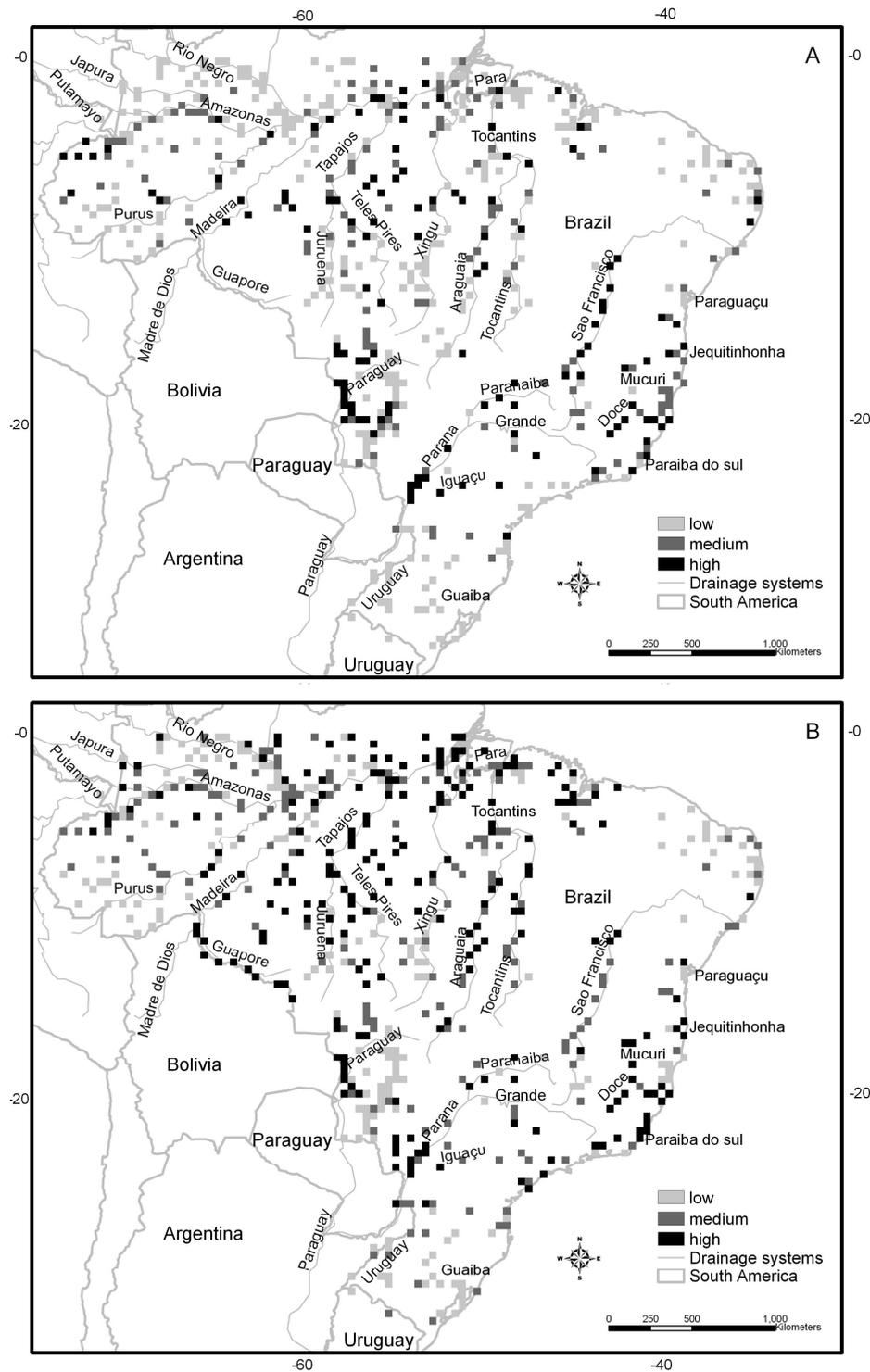


Figure 4. Predicted potential distribution of *L. fortunei* in the Brazilian river systems based on the GARP model calibrated to observed distributions within the Pantanal: (A) variable: $SI_{calcite}$, AUC= 0.82, Accuracy= 94%, Omission error= 5.8% (B) variables: calcium concentration, conductance, pH, water temperature, dissolved oxygen, AUC= 0.92, accuracy= 97%, Omission error= 2.9%. We considered the probability of establishment of *L. fortunei* as low (pale gray) when less than 5% of the models predicted the occurrence, medium (dark gray) when 5-50% of models predicted the occurrence and high (black) probability when more than 50% of models predicted the occurrence.

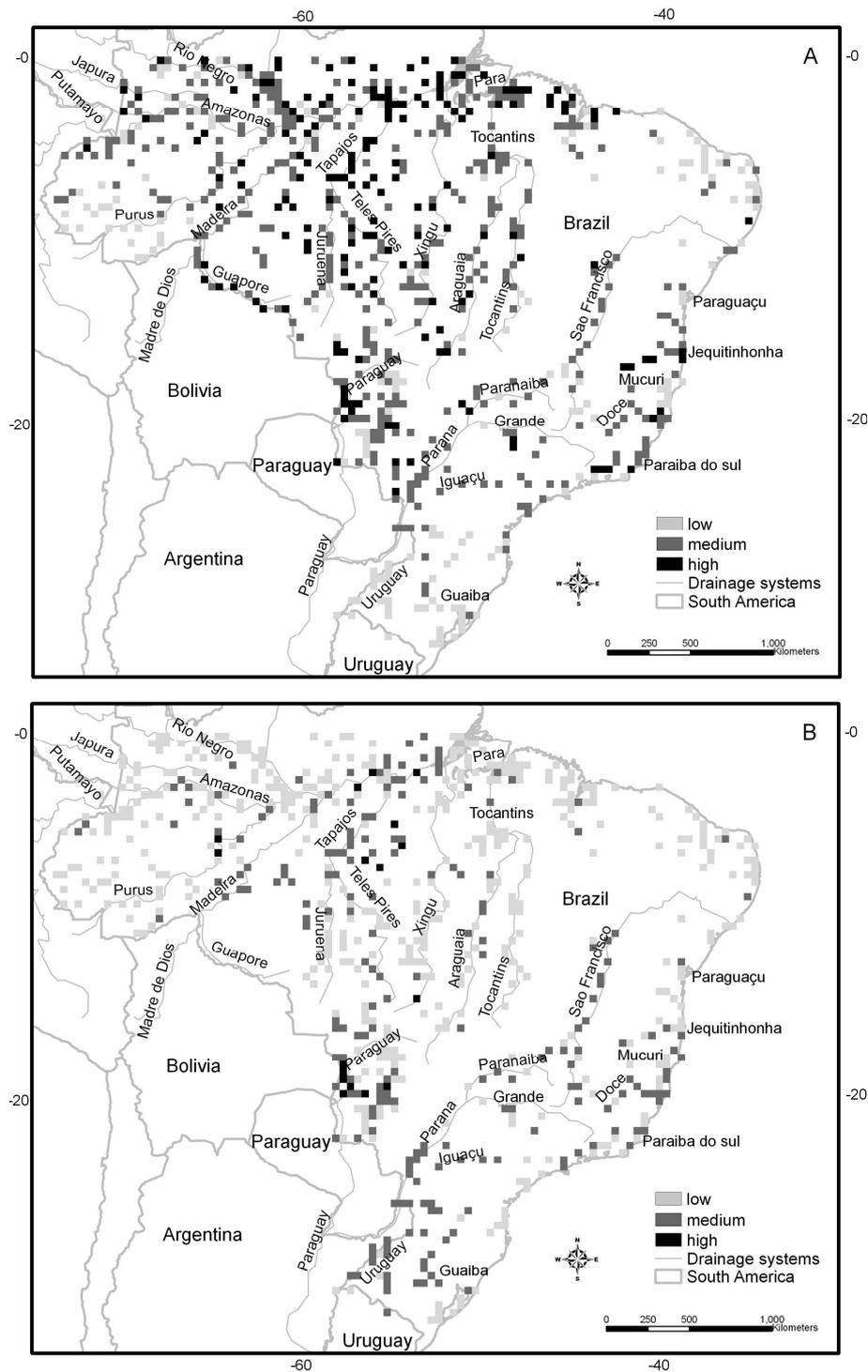


Figure 5. Predicted potential geographic distribution of the *L. fortunei* in the Brazilian river systems based on the Maxent model calibrated to observed distributions within the Pantanal: (A) variable: $SI_{calcite}$, $AUC=0.82$, threshold value= 3.0 (B) variables: calcium concentration, conductance, pH, water temperature and dissolved oxygen, $AUC=0.96$, threshold value= 5.5. Three shades are used to indicate the strength of the prediction. Maxent produces a continuous prediction with values ranging from 0 to 100, high in regions with better predicted conditions. The values are depicted here using light gray (between 0.0 and threshold value, low risk); dark gray (between threshold value and 50, medium risk); black (50-100, high risk).

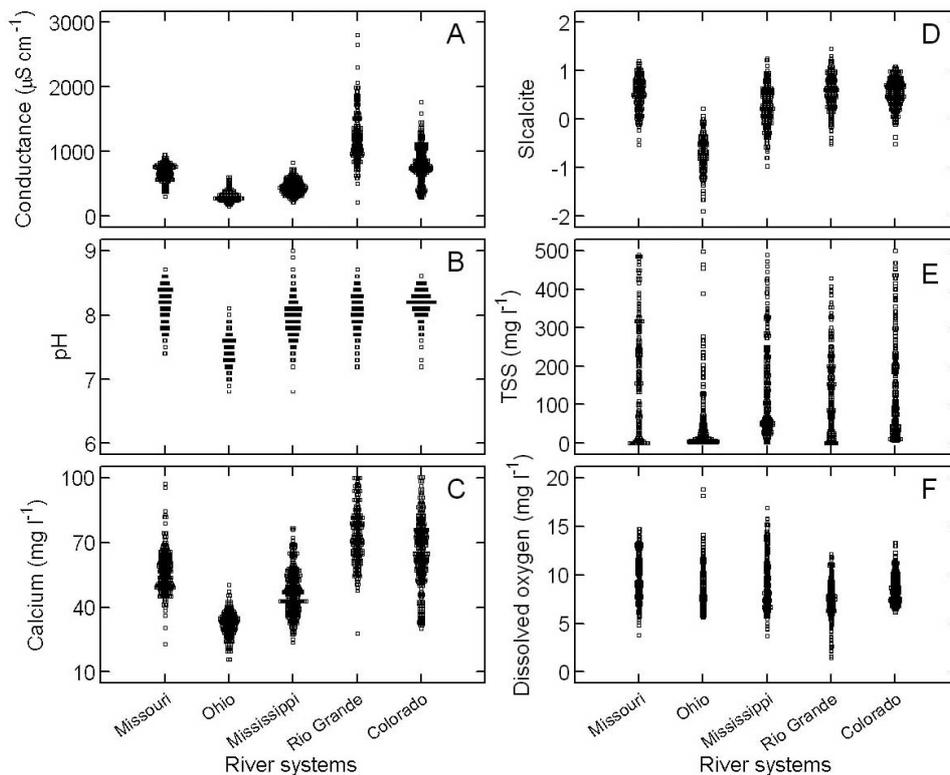


Figure 6. Ranges of conductance (A), pH (B), calcium concentration (C), SI_{calcite} (D), total suspended sediments (E) and dissolved oxygen (F) in the North American river systems. Limnological data set were provided by US Geological Survey's NASQAN program (description available at <http://pubs.usgs.gov/wri/wri014255/>). Data are monthly measures from 1995 to 2000 from 23 sites (1525 observations).

Limnological characterization of major North American river systems

Limnological characteristics of the North American rivers are depicted in Figure 6. Compared to the Paraguay River system (Figure 2), the North American rivers carry waters with higher conductance and dissolved calcium concentrations. Calcium in the North American rivers always exceeded 10 mg l^{-1} and pH was above 7.0. SI_{calcite} was usually in the range of -2 to 0, except for the Ohio River where SI_{calcite} was sometimes as low as -3. Dissolved oxygen was above 1.0 mg l^{-1} and concentrations of total suspended sediments often were higher than in Brazilian waters.

Water temperature as a limiting factor in North American rivers

In contrast to Ca concentrations and the SI_{calcite} , which do not appear limiting to the establishment of *L. fortunei* in the major North

American rivers, seasonally low water temperatures may present a limitation in northern regions. Winter water temperatures in the North American rivers range between 0 and 5°C in the upper Mississippi and its tributary the Missouri River (Figure 7). This low temperature can last for about 3 months in those rivers. In the Ohio River, the main eastern tributary of the Mississippi, minimum water temperatures are slightly higher than 5°C (Figure 8). The Colorado and Rio Grande rivers showed higher mean minimum temperatures, above 8°C during the winter season.

Our thermal tolerance assays using specimens of *L. fortunei* from the tropical Paraguay River provide an indication of how this species responds to low temperatures. Adult mussels survived up to 15 days at water temperatures of $0\text{-}1^{\circ}\text{C}$, although they were inactive during that time (Figure 9). Survival at $5\text{-}7^{\circ}\text{C}$ was $<50\%$ during the first 20 days, with a maximum survival of about 38 days. At temperatures above

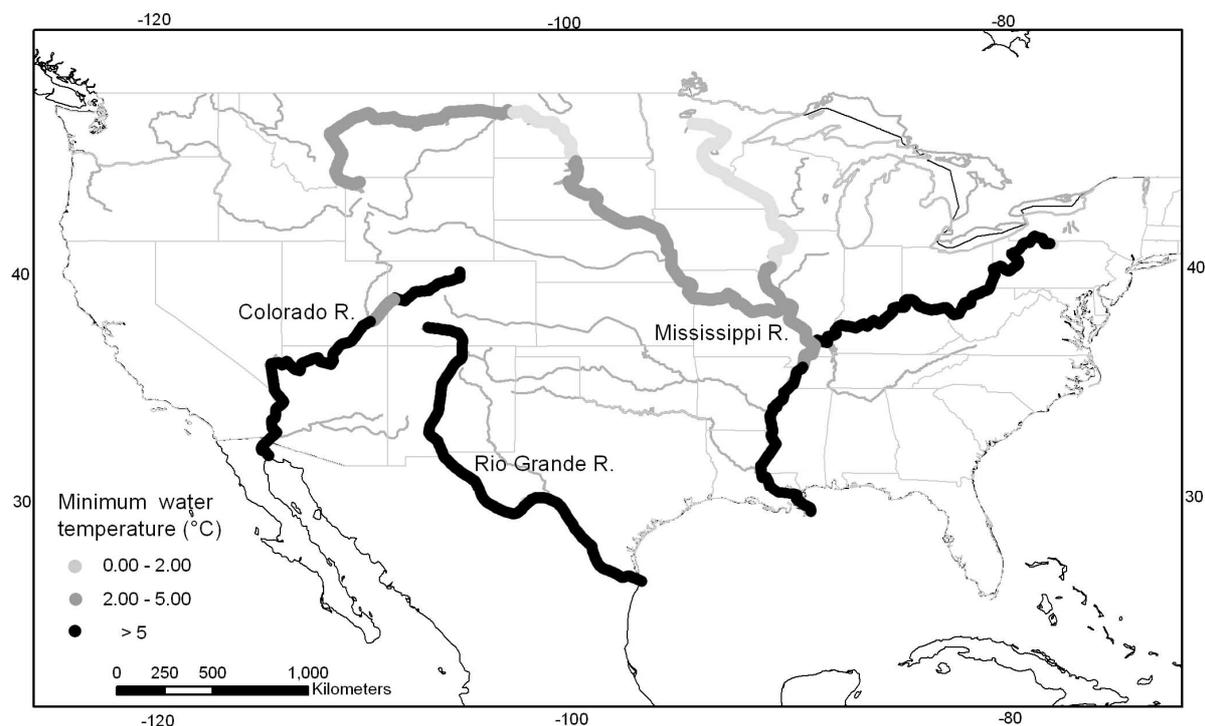


Figure 7. Spatial variation of water temperature (°C) in the Mississippi River system, including the Ohio and Missouri rivers, and in the Rio Grande and Colorado rivers. Data were taken from US Geological Survey's NASQAN program (description available at <http://pubs.usgs.gov/wri/wri014255/>) and represent mean of minimum water temperatures at 23 sites, from December to February, 1995 to 2000.

10°C mussels were active, and about 80% of them survived for the entire 30 days of the experiment. These data lend support to a threshold of ~5°C for protracted exposure of *L. fortunei* to low winter temperatures (i.e., weeks to months).

Discussion

Forecasting the expansion of L. fortunei in Brazilian river systems

In most places where *L. fortunei* occurs naturally (Morton 1975, Wang et al. 2007; Cai et al. 2008), or as an invader in the case of Brazilian river systems, the pH is above 6.4 and calcium concentrations are higher than 2.4 mg l⁻¹ (Morton 1975, 1977; Magara 2001; Cataldo and Boltovskoy 1999 and 2000). Nevertheless, in the Pantanal *L. fortunei* can occur in water where pH is around 6.0 and with low calcium

concentrations (e.g., 1.0 to 6.0 mg l⁻¹ in the Paraguay River) (Oliveira et al. 2006; Oliveira 2009). Considering the thresholds of tolerance of *L. fortunei* to diluted water as demonstrated by Deaton et al. 1989, we suggest that waters with less than 1.0 mg l⁻¹ of calcium concentration have low risk of *L. fortunei* establishment. Thus, most Brazilian river systems have medium to high probability of invasion based on calcium availability, with some exceptions located mainly in the Paraguay and Amazon River systems.

Both dissolved calcium concentrations and SI_{calcite} showed the potential for colonization of most Brazilian rivers. Neary and Leach (1992) predicted the expansion of the zebra mussel in Ontario (Canada) using calcium and pH, and the current distribution of zebra mussels after about 16 years (Zoltak 2008) matches the high risk areas indicated in that prediction. Models that simultaneously consider Ca and pH are

effectively similar to the SI_{calcite} since those are the most important controls on SI_{calcite} .

The minimum SI_{calcite} value observed in the Paraguay River system where *L. fortunei* is present was -4.0, in water with less than 1.0 mg l^{-1} of calcium. Most other Brazilian river systems had higher values of SI_{calcite} than the Paraguay River system, thus, it is likely that *L. fortunei* would have better conditions for calcification and therefore growth in most other Brazilian river systems compared to the Paraguay River system. Field observations are lacking for whether *L. fortunei* can become established in waters more undersaturated with respect to calcite than the Paraguay River value (i.e., SI_{calcite} below -4). During oxygen depletion events, when SI_{calcite} values were around -5.5, high mortalities were observed, although dissolved oxygen depletion occurred simultaneously and both low O_2 and low SI_{calcite} may have been stress factors (Oliveira 2009). Whereas both calcium and SI_{calcite} are good predictors for *L. fortunei* expansion in Brazil, they may not be good predictors for the major North American rivers, where calcium availability and calcification appear unlikely to be limiting factors.

Our study has focused on limiting factors for the occurrence of *L. fortunei* but conditions may act to limit the abundance of *L. fortunei* rather than preclude its establishment. Although there is no information about the effects of suspended sediments on *L. fortunei*, we have suggested that the combination of high concentrations of suspended sediments, low food availability, and high water velocity may be limiting factors for larval survival, settlement and growth in the Miranda River (upper Paraguay River basin) (Oliveira 2009). In rivers of the Pantanal, total suspended solids varied from 10 to 120 mg l^{-1} and chlorophyll *a* concentrations averaged less than $1.0 \text{ } \mu\text{g l}^{-1}$. High suspended sediment concentrations were observed mainly during the reproductive period of *L. fortunei* and may explain the low larval survival, perhaps together with water velocities exceeding 0.9 m s^{-1} .

L. fortunei can survive in turbid river waters with low food availability, and evidently it can use detrital organic carbon sources in addition to algae (Calheiros 2003; Sylvester et al. 2005). River-connected lakes in the Pantanal floodplain showed higher *L. fortunei* densities than the Paraguay River (Oliveira, unpublished). When positioned along river systems, these lentic water bodies may present improved conditions for

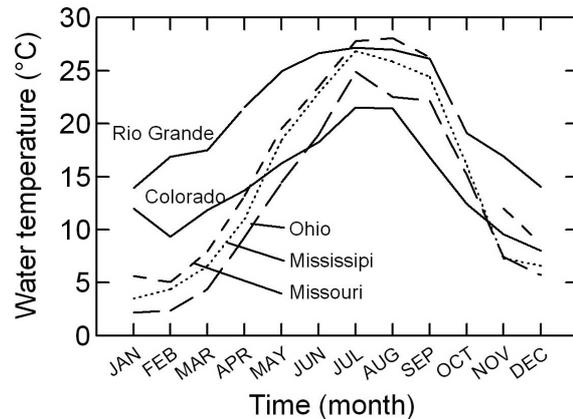


Figure 8. Mean seasonal patterns of water temperature ($^{\circ}\text{C}$) in North American river systems. Data were taken from US Geological Survey's NASQAN program (description available at <http://pubs.usgs.gov/wri/wri014255/>) from 1996 to 2000.

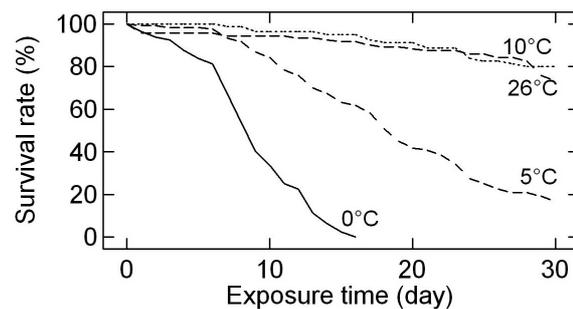


Figure 9. Survival rate of *L. fortunei* at temperatures of 0-1 $^{\circ}\text{C}$, 5-7 $^{\circ}\text{C}$, and 10-12 $^{\circ}\text{C}$. The lines show means of 6 replicates, except for 0 $^{\circ}\text{C}$ which had 9 replicates.

populations of *L. fortunei* and act as a source of larvae for downstream river reaches. Higher densities in lakes and a downstream decline in abundance of larval and settled mussels were also observed in small rivers in Ireland (Lucy et al. 2008).

GARP and Maxent models showed fewer regions where *L. fortunei* could become established compared to our conclusions based on the lower thresholds for dissolved Ca and SI_{calcite} . In the Brazilian river systems the ranges of conductance and calcium concentration were very broad compared to the area of occurrence of *L. fortunei* in the Pantanal, and some of these regions were predicted to be of low invasion risk

by GARP and Maxent but had medium to high risk based only on thresholds of calcium and SI_{calcite} . Additionally, both GARP and Maxent models failed to predict *L. fortunei* expansion in several relatively large-scale Brazilian river systems where calcium concentrations and SI_{calcite} values are favorable for its establishment. This suggests that these models might not be accurate when the calibration data used by the model to build the ecological niche do not span ranges similar to the data where predictions are made.

The GARP and Maxent models consistently predicted medium to high probability of *L. fortunei* occurrence in the Paraguay River, and most models predicted occurrence in the Paraná River, in both cases for sites where the species was already present. Furthermore, our predictions for the establishment of *L. fortunei* in Brazilian rivers were generally in agreement with analyses conducted by Kluza and McNyset (2005) over large spatial scales using mainly climatological data. According to our predictions sites located in the Amazon estuary as well as waters in the upper section of southern Amazon tributaries, which extend close to the Paraguay River system, such as the Tapajós, Teles Pires, and Araguaia rivers, have limnological conditions suitable for the occurrence of *L. fortunei*. However, they were predicted as low-risk areas by the Kluza and McNyset (2005) model. Low risk was also predicted for the São Francisco system and parts of the Paraná system, such as the Paranaíba River, in that model, contrasting with our prediction that these regions have medium to high potential of *L. fortunei* invasion.

Environmental variables that were input to the model and spatial scales of analysis are likely responsible for the differences between the Kluza and McNyset (2005) model results and our results. The Kluza and McNyset (2005) model was built using East Asian occurrence data and climatological and topographic variables for that region. The advantage of our method based on limnological variables is that it provides more detailed and waterbody-specific information; we can thus analyze each river system separately, and at finer spatial scales if desired, as we have done for the upper Paraguay River basin.

Considering the high potential risk in the vast majority of Brazilian river systems for the establishment of *L. fortunei*, management systems for invasions should focus on potential points of entry. The major South American

hydrographic basins including the Amazon, Orinoco, and Magdalena are readily accessible through their estuaries by ocean-going vessels, which make them highly vulnerable to invasions via ballast water releases (Boltovskoy et al. 2006). In central Brazil transport of mussels by recreational boaters and other human activities will also contribute to the range expansion of *L. fortunei*. Regions with high potential for invasion in the Paraguay and Paraná systems are close to those regions with high potential in the Amazon and São Francisco basins, and hence the risk of interbasin movement is great.

Predicting invasion potential of L. fortunei in North American rivers

If given the opportunity, *L. fortunei* probably will colonize North American waters, especially warmer waters in the southeast, potentially being introduced through ports along the Gulf of Mexico (Ricciardi 1998; Kluza and McNyset 2005; Karatayev et al. 2007). We can apply the knowledge of environmental tolerances of *L. fortunei* in the Pantanal, a region with relatively extreme environmental conditions, and elsewhere to project its potential distribution in representative major North American rivers. Low calcium availability and pH are unlikely to be limiting in these North American rivers because they are much higher compared to the native environments and Pantanal waters where *L. fortunei* is present.

In addition to the relatively low calcium and pH in some rivers of the Pantanal where *L. fortunei* has colonized, oxygen depletion events are common every year, particularly during the rising water phase of the annual inundation season, when river water first contacts the floodplains after the dry season (Calheiros and Hamilton 1998; Hamilton et al. 1997). The major North American rivers examined do not experience oxygen depletion events akin to those in Pantanal waters that can control the density of *L. fortunei*, although hypoxia is common during the summer in the Mississippi River estuary, and when combined with high temperature can provoke mortality of *Dreissena polymorpha* (Mihuc et al. 1999), even when dissolved oxygen concentrations are higher than 1.0 mg l⁻¹. Above its delta, oxygen concentrations in the Mississippi River are normally higher than 1.0 mg l⁻¹. The Rio Grande experiences some hypoxia also, but concentrations of oxygen remain above 1.0 mg l⁻¹.

High turbidity has been considered an important limiting factor for zebra mussel populations in large rivers like the Mississippi River, particularly in combination with high temperature (Alexander et al. 1994; Allen et al. 1999). Suspended sediments in the three major North American rivers considered here were usually higher compared to the rivers of the Pantanal. In the Mississippi River, where mean winter water temperatures are above 5°C, concentrations of total suspended solids ranged between 51 and 1,360 mg l⁻¹, and DOC concentrations were low compared to the Paraguay River. This combination of high suspended sediments and low food availability could limit the abundance of *L. fortunei* where the temperature is not a limiting factor.

L. fortunei lives in tropical waters in the Pantanal, where water temperatures of around 30°C last about 7 months of the year, and minimum temperatures are usually above 17°C (Oliveira et al. 2006; Oliveira 2009). *L. fortunei* is able to survive and undergo larval development in temperatures up to 33°C in the Pantanal wetland (Oliveira 2009). Whereas high water temperature limits dreissenid mussels in North America, low water temperature may be more likely to be the key environmental variable determining distribution patterns of *L. fortunei* in North American rivers (Ricciardi 1998), although Karatayev et al. (2007) suggested that the lower thermal limits of *L. fortunei* may resemble those of the invasive dreissenids that flourish in the temperate climate of the Laurentian Great Lakes region (US and Canada). Thermal tolerance depends on duration of exposure as well as maximum or minimum temperatures. Observations in the literature indicate that *L. fortunei* can exist at high densities in waters that fall as low as 5–8°C (Japan: Magara et al. 2001; Goto 2002), although in both of these cases the season of low water temperatures was short, lasting about 2 months; or near 0°C (South Korea: Choi and Kim 1985; Choi and Shin 1985, reviewed in Karatayev et al. 2007).

Based on our water temperature tolerance assays, the probability of *L. fortunei* establishment would be low in water bodies with temperatures below 5°C for protracted periods. In the Upper Mississippi the minimum winter temperature is about 0°C, with around 3 to 4 months of temperatures lower than 5°C. It is possible that this protracted cool period is too long for adult *L. fortunei* survival. The probabi-

lity of establishment and survival is greater in the Mississippi River below its confluence with Ohio River (Mississippi River at Thebes, IL), as well as in the Colorado River and Rio Grande, where the minimum winter water temperature is above 5°C.

The potential distribution of *L. fortunei* overlaps the current distribution of the corbiculid and dreissenid mussels in North America and moreover further extends into waters that are warmer, lower in calcium, and subject to oxygen depletion events. Hence the ecological and economic impacts of the spread of *L. fortunei* are potentially more far-reaching than those of the dreissenids, and efforts to prevent its introduction and contain its spread are well justified.

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