Modeling the potential distribution of the invasive golden mussel *Limnoperna fortunei* in the Upper Paraguay River system using limnological variables

Oliveira, MD.[a]**, Hamilton, SK.*[b]*, Calheiros, DF.*[a]*, Jacobi, CM.*[c] *and Latini, RO.*[d]

[a]Embrapa Pantanal, Rua 21 de Setembro, 1880, CP 109, CEP 79320 900, Corumbá, MS, Brazil

[b]W.K. Kellogg Biological Station, Department of Zoology, Michigan State University, 3700 E. Gull Lake Drive, Hickory Corners, MI 49060-9516 USA

[c]Universidade Federal de Minas Gerais – UFMG, Av. Antonio Carlos, 6627, CEP 31270-901, Belo Horizonte, MG, Brazil

[d]Centro Universitário Metodista Izabela Hendrix, Rua da Bahia, 2020, Praça da Liberdade, CEP 30160-012, Belo Horizonte, MG, Brazil

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*e-mail: mmarcia@cpap.embrapa.br

(With 6 figures)

Abstract

The invasive golden mussel, *Limnoperna fortunei* (Dunker, 1857), was introduced into the La Plata River estuary and quickly expanded upstream to the North, into the Paraguay and Paraná rivers. An ecological niche modeling approach, based on limnological variables, was used to predict the expansion of the golden mussel in the Paraguay River and its tributaries. We used three approaches to predict the geographic distribution: 1) the spatial distribution of calcium concentration and the saturation index for calcium carbonate (calcite); 2) the Genetic Algorithm for Rule-Set Production (GARP) model; and the 3) Maximum Entropy Method (Maxent) model. Other limnological variables such as temperature, dissolved oxygen, pH, and Total Suspended Solids (TSS) were used in the latter two cases. Important tributaries of the Paraguay River such as the Cuiabá and Miranda/Aquidauana rivers exhibit high risk of invasion, while lower risk was observed in the chemically dilute waters of the middle basin where shell calcification may be limited by low calcium concentrations and carbonate mineral undersaturation.

Keywords: golden mussel, ecological niche modeling, Paraguay River, Pantanal wetland.

Modelagem da distribuição potencial do mexilhão dourado *Limnoperna fortunei* na Bacia do Alto Rio Paraguai usando variáveis limnológicas

Resumo

A espécie invasora mexilhão dourado, *Limnoperna fortunei* (Dunker, 1857), foi introduzida na bacia do Rio da Prata e rapidamente se expandiu em direção ao norte, nos rios Paraguai e Paraná. A modelagem de nicho ecológico com base em variáveis limnológicas foi utilizada para prever a expansão do mexilhão dourado na bacia do Alto Paraguai. Foram usados três métodos para prever a distribuição espacial do mexilhão dourado: 1) a distribuição espacial da concentração de cálcio e o índice de saturação do carbonato de cálcio (calcita); 2) a modelagem utilizando o algoritmo *Genetic Algorithm for Rule-Set Production* (GARP); e 3) a modelagem usando o *Maximum Entropy Method* (Maxent). Outras variáveis como temperatura da água, oxigênio dissolvido, pH e sólidos totais suspensos também foram utilizadas na modelagem. Importantes tributários do Rio Paraguai como Cuiabá e Miranda exibem alto risco de invasão, e menores riscos foram observados para águas mais diluídas na parte média da bacia, onde a calcificação das conchas pode ser limitada pela baixa concentração do cálcio e dos carbonatos minerais abaixo do nível de saturação.

Palavras-chave: mexilhão dourado, modelagem de nicho ecológico, Rio Paraguai, Pantanal.

1. Introduction

The invasive golden mussel, *Limnoperna fortunei* (Dunker, 1857), was introduced into the La Plata River estuary and quickly expanded upstream to the North, into the Paraguay and Paraná rivers. The upstream colonisation of *Limnoperna fortunei* in the Paraguay River system is mainly facilitated by the regular boat traffic along the Paraguay-Paraná waterway, which includes barge trains that readily transport organisms (Oliveira et al., 2006). Dispersal from the main river channel to the tributaries is slower due the lower boat traffic (Boltovskoy et al., 2006). According to these authors *L. fortunei* expands its range via two basic modes of transport: a gradual dispersion from a localised epicentre and a 'jump dispersal'. Both processes will ensure that *L. fortunei* expands far beyond the Paraná-Paraguay systems in Brazil, although it may take more time than in the Paraguay River.

Considering that the means of introduction are present in most environments, the expansion of invasive species might be predicted on the basis of their ecological requirements and characteristics of the receptor environment, using the ecological niche theory described in Peterson and Vieglais (2001). Modeling based on ecological niches may allow identification of environments potentially suitable for a new species. Predictive models are developed through a three-step process: 1) modeling niches in ecological space, 2) evaluating these niche models based on native distributions, and 3) projecting the models to regions that could be invaded (Peterson and Vieglais, 2001).

Ecological niche modeling can be used to assess potential geographic distributions of non-native species when a good database is available (Peterson and Vieglais, 2001; Peterson, 2003). Two examples are the prediction of expansion of *Dreissena polymorpha* in North America (Bossenbroek et al., 2007; Drake and Bossenbroek, 2004) and the expansion of *L. fortunei* globally (Kluza and McNyset, 2005). These models have shown good results for large spatial scales using environmental layers from climatological or geological and topographic data; however, they do not provide detailed information at small geographic scales for aquatic species.

For smaller scale distributions, such as within a river system, 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).

The aim of this study was to forecast the geographic expansion of *L. fortunei* at a regional spatial scale, the Upper Paraguay River basin, applying physical and chemical aquatic variables that are not commonly used in models over broader spatial scales. The advantage of modeling the potential distribution is that the possibility of an invasion can be assessed before the actual introduction of the species (Peterson and Vieglais, 2001). Results from this study can be used to establish priority areas to monitoring, management and biodiversity conservation.

2. Material and Methods

2.1. Study area

The Upper Paraguay River Basin (UPB), where most of the study was conducted, covers about 496,000 km^2 along the western border of Brazil. Our study area included the Paraguay River and its major tributaries (Figure 1), and connected lakes, in the Pantanal wetland.

The Paraguay River, the main channel draining the basin, runs from North to South collecting the waters of large tributaries such as Jauru, Cabaçal, and Sepotuba rivers along the right margin, and the Cuiabá (with its tributaries São Lourenço and Piquiri rivers), Taquari, Negro and Miranda (with its tributary Aquidauana) along the left margin. Also on the right margin, there is an extensive series of river-connected lakes surrounded by higher land (Hamilton et al., 1997; PCBAP, 1997).

Most Pantanal waters are low in dissolved ions and slightly acidic. More alkaline waters occur in the Southernmost areas of the Pantanal where the Miranda and Apa basins are located, as a result of carbonate rocks present in the upland watersheds (Hamilton et al., 1997). Deforestation and agricultural activities in the upland areas surrounding the Pantanal increase sediment inputs along the river courses, and introduce nutrients and pesticides to

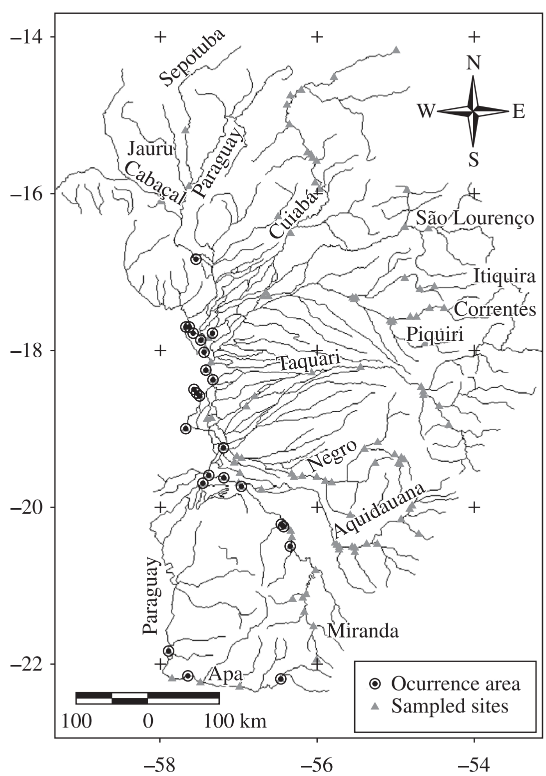


Figure 1. Current distribution of *L. fortunei* in the UPB as of 2008 (full triangle). Open circles represent locations sampled for limnological variables. UPB drainage map source: ANA (National Agency of Water), scale: 1:100.000. Map built using Arc View 3.2 software.

the water, which can reach the floodplains (Miranda et al., 2008). In the floodplain extensive cattle ranching is the main economic activity, followed by tourism including sport fishing, and professional fishing. Navigation in the Paraguay-Paraná waterway is another important economic activity in the Paraguay River, and can connect five countries in South America.

2.2. Data collection and analysis

The potential distribution of *L. fortunei* in the UPB was predicted in three different ways: through an exploratory analysis based on the spatial distribution of calcium concentrations and the saturation index of calcium carbonate ($SI_{calcite}$), and by applying two niche models that have been used to predict alien species invasions: Genetic Algorithm for Rule-set Prediction (GARP) and Maximum Entropy Method (Maxent). The rationale for using $SI_{calcite}$ is that calcium concentrations are not the only influence on shell calcification; pH and alkalinity are also important and the calcite mineral saturation index integrates these variables to provide an indicator of the overall thermodynamic tendency for carbonate dissolution or precipitation.

The GARP model describes environmental conditions under which the species should be able to maintain viable populations. For input, GARP uses a set of point localities where the species is known to occur and a set of geographic layers representing the environmental parameters that might limit the species' capabilities to survive (Stockwell and Noble, 1992; Peterson and Cohoon, 1999; Stockwell and Peterson, 1999). We used GARP best subsets to find the best fitting models according to Anderson et al. (2003) from the openModeller Desktop (http://openmodeller.sf.net and http://openmodeller.cria.org.br/wikis/omgui).

The Maxent algorithm estimates the geographic distribution of species from point locality data by finding the probability distribution of maximum entropy (i.e., that is most spread out, or closest to uniform), subject to a set of constraints that represent incomplete information about the target distribution (Phillips et al., 2006). The software is available at http://www.cs.princeton.edu/~chapire/maxent, version 3.0.4 beta.

All predictions were made using the following limnological variables: water temperature, dissolved oxygen, conductance, pH, calcium concentration, Total Suspended Solids (TSS), and a calcite saturation index ($SI_{calcite}$). $SI_{calcite}$ was calculated based on water temperature, pH, conductance, total alkalinity (Gran, 1952), and dissolved calcium, following American Public Health Association (2005). The $SI_{calcite}$ is plotted on a log scale where zero indicates thermodynamic equilibrium with respect to $CaCO_3$, a positive index indicates supersaturation, and a negative index indicates undersaturation. We used Total Suspended Solids (TSS) in the UPB as a variable related to the inorganic turbidity. Additionally chlorophyll-*a* was used as an indicator of food availability (algal biomass in suspension) for this mussel species, which is a filter-feeder.

Not all variables have established limits for *L. fortunei*. We considered the minimum pH, calcium and $SI_{calcite}$ requirement for *L. fortunei* development to be 6.0, 1.0 $mg.L^{-1}$ and –4.0, respectively. These limits were established based on Ricciardi (1998) and Oliveira et al. (2010a). Above these limits we considered that waters have medium to high risk to support *L. fortunei* invasion and below these limits the risk of invasion is low.

Data used to model the potential distribution in the UPB were taken from the Embrapa Pantanal database (Long Term Ecological Program – PELD/CNPq), and the Secretariats of the Environment of the states of Mato Grosso and Mato Grosso do Sul. Data from nine sampling sites in the Paraguay River, 114 sites in the most important tributaries of the Paraguay River basins, and one site in each of seven connected lakes were used. We used limnological samples taken only during the low water phase, because in this phase the rivers are restricted to the main channel and have less interaction with the floodplain. We calculated mean values since most of the sites have several years of sampling (4 to 10 years). To understand the effects of oxygen depletion events on *L. fortunei* dispersion we monitored daily the dissolved oxygen and pH in the Miranda and Cuiabá rivers during oxygen depletion events in 2007.

Environmental layers (georeferenced chemical and physical data) and records of presence (latitude/longitude) of *L. fortunei* in the Pantanal wetland were used to model its potential distribution in the UPB. Environmental layers were built using the software Arc View 3.2 (Environmental Systems Research Institute, 1999). A drainage map of the UPB was obtained from the National Water Agency (Agência Nacional de Águas). In order to generate continuous pixel lines along the rivers we interpolated values every 10 km in the UPB from the 130 primary sample sites, as explained in Latini (2006), totalling 943 geographic points. Grid cells of 0.07 degrees were used in the case of UPB. From a total of 130 sites in the UPB, 24 sites had occurrence records located in the Pantanal wetland (Figure 1). We used 50% of this data for training and 50% for validation in both GARP and Maxent models.

To test the performance of the GARP and Maxent models we used the Receiver Operating Characteristic (ROC) analysis, which characterises the performance of a model at all possible thresholds by a single number, the Area Under the Curve (AUC) (Phillips et al 2004, 2006). The AUC can be interpreted as the probability that a model set correctly predicts presence in a randomly selected grid cell. It plots omission error against threshold, and predicted area against threshold. 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 AUC above 0.75 were considered in this study. Our goal was not to compare outputs between GARP and Maxent, since the strength of their predictions cannot be compared directly (Philips et al., 2006), but to interpret results from each one separately according to our experience with *L. fortunei* in the UPB.

To assess the soundness of the predictions we also used omission error and accuracy from GARP, and a threshold value in Maxent. The cumulative threshold value is given in the Maxent output. It is a balance between training omission and predicted area, and threshold value (Phillips et al., 2006). Values below the thresholds were considered with low conditions of *L. fortunei* occurrence. The potential absence was included in the low risk category, because of the incertanties about environmental conditions under which *L. fortunei* should not be able to maintain viable populations.

3. Results

3.1. Current L. fortunei distribution in the Upper Paraguay basin

The current occurrence area of *L. fortunei* in the UPB is shown in Figure 1. *L. fortunei* is restricted to the Paraguay River main channel, and adjacent inundation area, including connected lakes, and the lower and middle portions of several left-bank tributaries (Cuiabá, Miranda and Apa rivers). Except for the Cuiabá River, where the population density is very low and colonisation may be at an earlier stage, in all other places the populations appear to be fully established.

3.2. Limnological characterisation of the Upper Paraguay basin

In general, the UPB waters are dilute in dissolved ions as exemplified by the Paraguay River, where conductance was around 50-60 µS.cm^{-1}. More dilute waters were found in the central part of the basin: 3.0 to 19.0 µS.cm^{-1} in the São Lourenço, Correntes and Piquiri rivers (Cuiabá basin), 10.0 to 20.0 µS.cm^{-1} in the Sepotuba, and ~25.0 µS.cm^{-1} in the Taquari and Negro rivers.

Some of these rivers exhibited minimum calcium concentration low as 0.0 mg.L^{-1} (Figure 2a), averaging 2.0 mg.L^{-1}. They also had the most unsaturated water related to calcium carbonate: $SI_{calcite}$ was around -5.4 to -6.0, while in the Paraguay River the values were between –2.0 and –4.0 (Figure 2b). In general the calcium concentration in the Paraguay River is above 3.0 mg.L^{-1} (Figure 2a), but may be as low as 1.0 mg.L^{-1}.

In the Upper Cuiabá River, the major tributary of Paraguay River, conductance was between 60 and 100 µS.cm^{-1}. This river water can be diluted with floodplain waters in the lower reaches within the Pantanal. In the floodplain area, the Cuiabá River receives water from the São Lourenço and Piquiri rivers, where mean conductance was 38.0 µS.cm^{-1}. Calcium concentrations in the lower Cuiabá River were between 3.0 and 4.0 mg.L^{-1}, but were sometimes as low as 1.2 mg.L^{-1}.

Some tributaries of the Miranda and Apa rivers carry water with conductance as high as 400-600 µS.cm^{-1}. In these rivers, minimum calcium values were above 5.0 mg.L^{-1} and reached around 60 mg.L^{-1} (Figure 2a). In the lower section of the Miranda River conductance was between 84 and 178 µS.cm^{-1}.

Waters with less extreme undersaturation of calcium carbonate (Figure 2b) were observed in the Upper Cuiabá, Miranda/Aquidauana and Apa river, where $SI_{calcite}$ ranged between –3 and 0.7. The most unsaturated waters were found in the São Lourenço and Negro river systems.

The mean pH in the UPB is predominantly between 6.0 and 7.0 (Figure 2c). Although the pH is above 6.0 in the upper section of the tributaries it can be lower in the lower section (i.e., 5.0 in the lower Negro River, 5.1 in the lower Cuiabá River, and 6.2 in the lower Taquari).

In general, rivers in the UPB carry high suspended sediment concentrations during the low-water phase, as for example in the Taquari, Miranda, São Lourenço, and Vermelho rivers. The mean concentrations of total suspended solids in the tributaries reached 290 mg.L^{-1}, higher than in the Paraguay River, where the suspended solids oscillated around 50 mg.L^{-1} (Figure 2d). In the floodplain reaches these tributaries show some reduction in TSS concentrations. Concentrations of chlorophyll-*a* in the UPB rivers, in general, were low, with means around 1.0 to 2.0 µg.L^{-1}.

Water temperatures observed in the UPB reflect the tropical climate, oscillating between 16.0 and 34.5 °C. Minimum temperatures were recorded between May and July and maxima between October and March. During much of the year, dissolved O_2 concentrations were around 6.0 mg.L^{-1} or 50-60% of atmospheric equilibrium in the Paraguay basin. Rivers located in the upland areas had minimum dissolved oxygen concentrations above 2.0 mg.L^{-1}; however oxygen can fall as low as 0.0 mg.L^{-1} during rising water in the lower sections where floodplain influence is greater. Oxygen depletion events, defined as oxygen concentrations falling below 2.0 mg.L^{-1} (Oliveira, et al. 2010b), were recorded in the Paraguay River floodplain including the lower portion of its tributaries, such as the Miranda and Cuiabá rivers (Figure 3). These events were observed once a year, and might last from about a week to more than one month. Dissolved oxygen dropped from 6.0 to 0.0 mg.L^{-1} and pH fell as low as 6.2 in the Miranda River. In the Cuiabá River dissolved oxygen fell from 6.5 to 0.0 mg.L^{-1} and pH from 6.5 to 5.1 (Figure 3). Oxygen depletion events

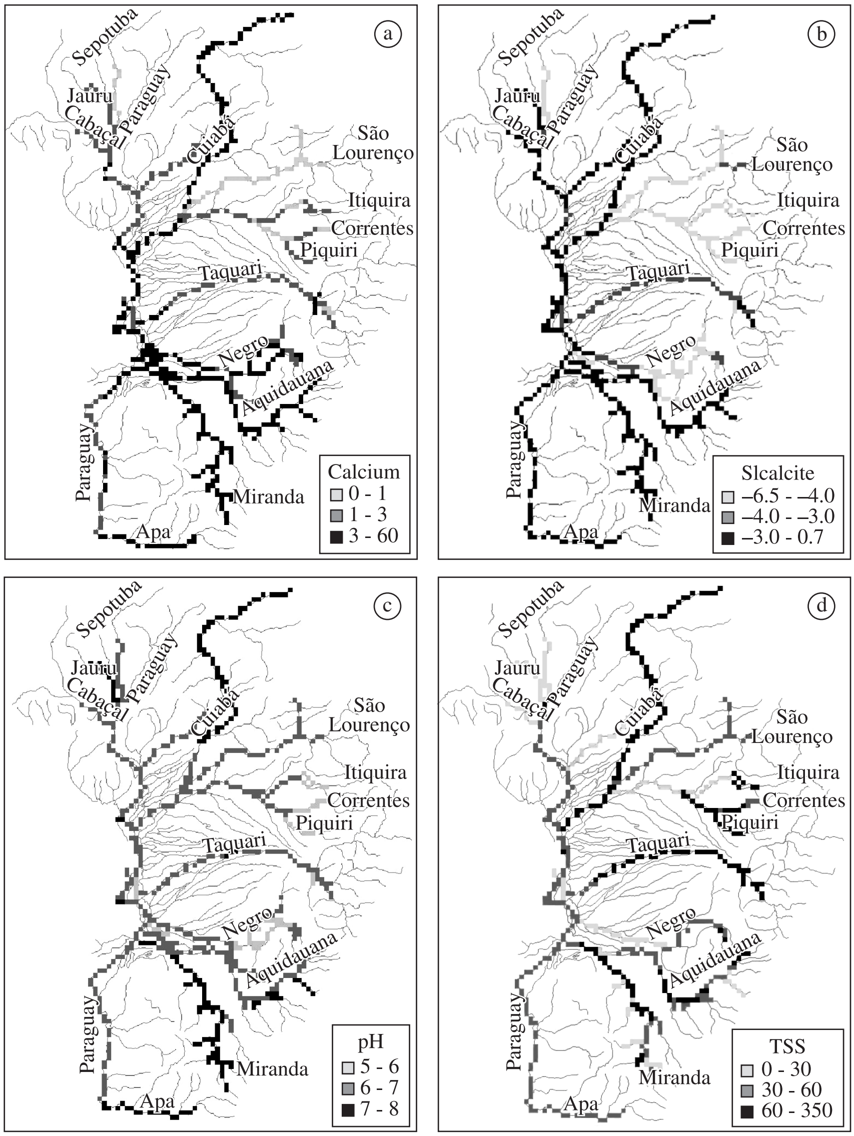


Figure 2. Spatial variation of limnological variables in the UPB. a) calcium concentration ($mg.L^{-1}$), b) $SI_{calcite}$, c) pH, and d) TSS ($mg.L^{-1}$). Data are means for samples collected during the low water phase.

occur from February to May when temperature is around 30.0 °C, and can reach 34.0 °C, and usually stop when temperature decreases, normally after May.

3.3. Forecasting the expansion of L. fortunei in the Upper Paraguay basin

Most water in the UPB has medium to high risk of invasion by *L. fortunei* according to calcium concentration predictions (Figure 4a). High risk of occurrence was correctly predicted in the regions where *L. fortunei* is present, such as Paraguay River, connected lakes and lower Miranda River. Additionally, this method also predicted high risk to regions where *L. fortunei* was not recorded yet, such as Cuiabá, Negro, Upper Miranda/Aquidauana and Upper Apa rivers. There is medium risk that the species becomes established in the Northern UPB, including the Paraguay River above the Pantanal and its tributaries the Cabaçal and Jauru rivers, and in the middle of UPB, including the Taquari and Negro river tributaries. The São Lourenço and Sepotuba rivers have a low invasion risk. Prediction based on $SI_{calcite}$ showed more regions with high invasion risk than did predictions based on calcium concentration alone, mainly in the Paraguay River, Cabaçal, Jauru and Taquari rivers, but with lower risk in the Negro River (Figure 4b).

Results from the GARP best subset models using $SI_{calcite}$ (Figure 5a) or combined limnological variables

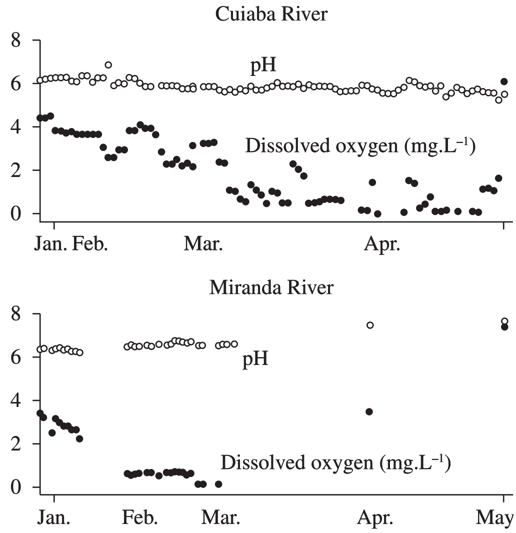


Figure 3. Short-term variation (daily sampling) of dissolved oxygen and pH during oxygen depletion events in the Cuiabá River (top graph) and Miranda River (bottom graph) in 2007.

(Figure 5b) were in agreement with individual calcium and $SI_{calcite}$ predictions described above, suggesting medium to higher risk for most of the rivers. Specifically, the $SI_{calcite}$ GARP model showed a close resemblance to the $SI_{calcite}$ threshold-based forecast, indicating greater risk for the Cabaçal, Jauru, Cuiabá, and Aquidauana rivers as well as portions of the Upper Paraguay River compared with the combined variables GARP model. Both models generally predicted low risk for São Lourenço basin, Taquari, and Negro rivers.

Maxent does not produce probabilities as an output. Rather, it shows regions with better predicted conditions, ranging from 0 to 100 (Figure 6). Maxent models using either $SI_{calcite}$ (Figure 6a) or combined limnological variables (Figure 6b) also indicated that the Cuiabá, Miranda, Aquidauana and Apa rivers, the upstream section of Paraguay River, and the Jauru and Cabaçal rivers present better conditions for *L. fortunei* establishment. Differently from other models, the Maxent combined variables results (Figure 6b) considered the São Lourenço River to have good conditions to support *L. fortunei* and Maxent $SI_{calcite}$ model indicated that Taquari River also has favorable conditions to *L. fortunei* colonisation. Considering results from all models most of the Paraguay basin has conditions for *L. fortunei* development.

4. Discussion

As *L. fortunei* expands to new areas our understanding about its tolerance to environmental variables improves. Before *L. fortunei* invasion in the Pantanal wetland it was

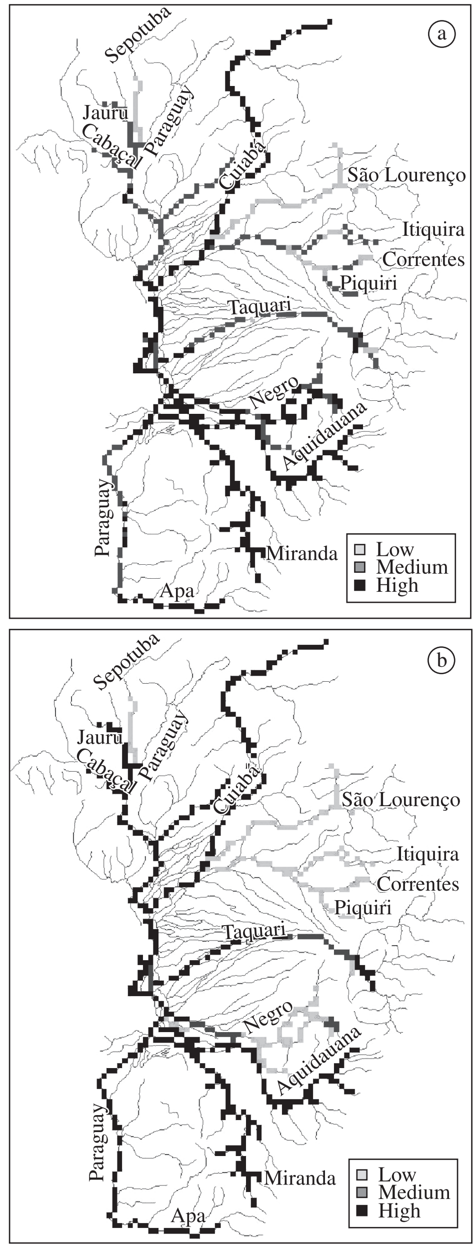
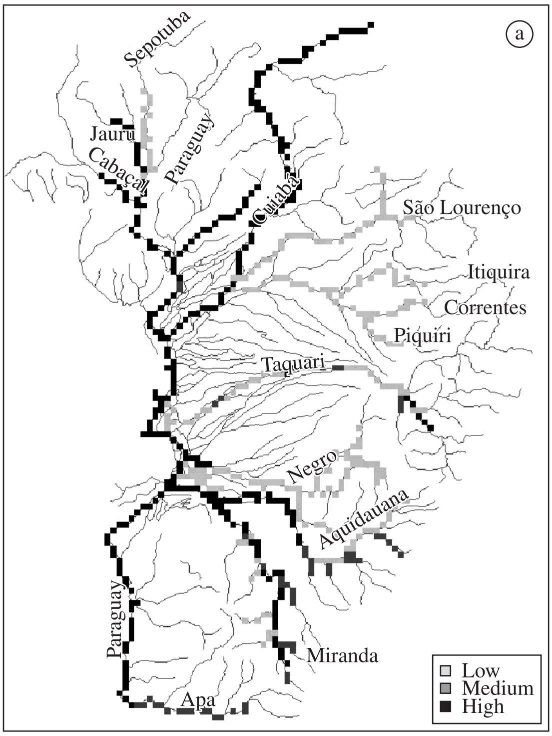


Figure 4. Potential distribution of *L. fortunei* in the UPB based on thresholds of calcium concentration a) and $SI_{calcite}$ b). We considered low risk of *L. fortunei* occurrence in rivers where mean of calcium concentration was <1.0 mg.L^{-1} and $SI_{calcite}$ was <–4.0, medium risk between calcium 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.

believed that *L. fortunei* was only able to become established in water with pH above 6.4 and calcium concentrations higher than 2.4 mg.L^{-1} (Morton, 1975; Magara et al., 2001; Cataldo and Boltovskoy, 1999, 2000). However, in the Paraguay River *L. fortunei* populations have become established in waters of lower pH, around 6.0, and lower



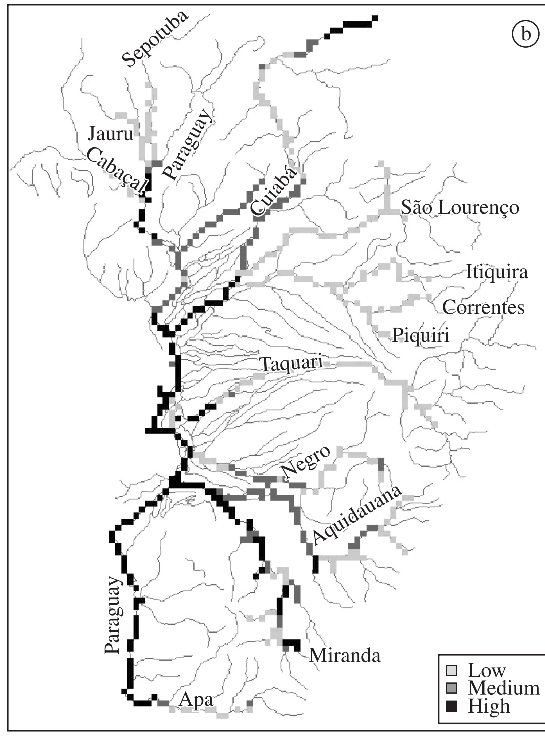


Figure 5. Predicted potential geographic distribution of the *L. fortunei* in the UPB based on the GARP model using Pantanal occurrence data. a) variable: $SI_{calcite}$; AUC = 0.75, accuracy = 95.6%, omission error = 4.3%; b) variables: calcium concentration, conductance, pH, water temperature, dissolved oxygen and total suspended solids; AUC = 0.85, accuracy = 87.5%, omission error = 12.5%. We considered the risk 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) risk when more than 50% of models predict the occurrence.

calcium concentrations, ranging from 0 to 6.0 $mg.L^{-1}$ (Oliveira, et al. 2010a). Results from Deaton et al. (1989) suggested that mussels might live in such dilute waters, producing byssal attachments and filtering after incubation for 1 week in deionised water.

Based on calcium concentration most rivers in the Paraguay basin have medium to high probability of *L. fortunei* invasion, and some additional rivers with calcium less than 1.0 $mg.L^{-1}$ may have low risk but some chance to support populations (Deaton et al., 1989; Oliveira, 2009). $SI_{calcite}$ provided results comparable but not identical to those predicted by calcium concentration. $SI_{calcite}$ is an index of the potential for calcification by *L. fortunei*, integrating alkalinity, calcium and pH, considered important habitat indicators by Ramcharan et al. (1992) based on studies of *D. polymorpha* in North America. We can conclude that waters that have more than 1.0 $mg.L^{-1}$ of calcium and $SI_{calcite}$ above –4 have potential to *L. fortunei* establishment, with greater likelihood of abundant populations in waters well above these thresholds.

The advantage of our prediction based on limnological variables is that it provides more detailed and specific information for specific rivers and reaches within rivers. Until now, niche models like GARP and Maxent have employed broad-scale indicators such as climate rather than limnological variables like pH and calcium to predict the spread of aquatic invasive mollusks, although these variables have been used in other predictive studies (Ramcharan et al., 1992; Neary and Leach, 1992). Results from our GARP and Maxent models showed that regions indicated as having medium to high risk to support *L. fortunei* in the Paraguay, Cuiabá, Miranda/Aquidauana, and Apa rivers are coincident with those where calcium concentration is higher than 1.0 $mg.L^{-1}$, while regions with low risk are those where calcium concentration is below this threshold, corroborating the idea that these models might give good results using limnological variables. Predictions from alternative models were consistent since all predicted medium to high probability in the Paraguay River where the species is present.

The agreement between threshold-based models using calcium and $SI_{calcite}$ and mathematical modeling (GARP and Maxent) suggests that both empirical models can be used to forecast *L. fortunei* expansion as an alternative to

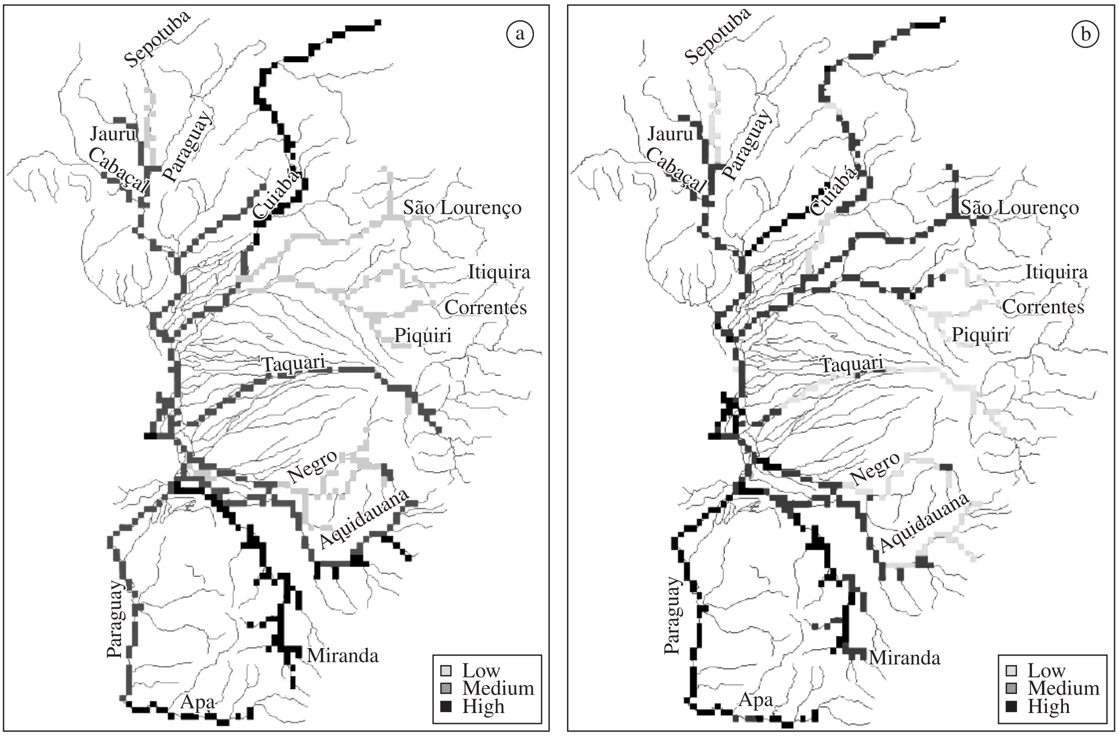


Figure 6. Predicted potential geographic distribution of *L. fortunei* in the UPB based on the Maxent model using Pantanal occurrence data. (a) variable: $SI_{calcite;}$ AUC = 0.77, threshold value = 4.6; (b) variables: calcium concentration, conductance, pH, water temperature, dissolved oxygen and total suspended solids; AUC = 0.81, threshold value = 5.6. Maxent produces a continuous prediction with values ranging from 0 to 100, increasing to regions with better predicted conditions. We used three gray hues to indicate the strength of the prediction. The values are divided using light gray (between 0.0 and threshold value, low risk), dark gray (between threshold value and 50, medium risk) and black (50-100, high risk). More information about the threshold value was provided in the methodology section.

mathematical models that are more complex. The advantage of calcium and $SI_{calcite}$ threshold-based prediction is that it is easy to use, although the calculation of $SI_{calcite}$ requires several limnological measurements. It is not necessary to have specific software like GARP and Maxent, or use GIS to predict the *L. fortunei* occurrence in a particular water body. This is particularly efficient for prediction at small spatial scales (Oliveira, 2009).

Water temperature was considered not limiting in the Paraguay basin because it is within the tolerance range for *L. fortunei*, but seasonal temperature changes might control the reproductive activity (Oliveira, 2009). Dissolved oxygen needs to be considered as a limiting factor during oxygen depletion events in the Paraguay River and its floodplain, although within the river channels during low water it is not a limiting factor.

Negative effects of high inorganic turbidity were observed in zebra mussel populations in large rivers like the Mississippi River system, particularly when combined with high temperature (Alexander et al., 1994; Allen et al., 1999). The combination of high concentration of suspended sediments, low food availability and high water velocity might be a limiting factor in the Miranda River, especially to larval settlement (Oliveira et al. 2010a). In the UPB, the Cuiabá, Miranda/Aquidauana and Apa rivers are the systems with highest potential of *L. fortunei* occurrence, however, these rivers present relatively high concentrations of total suspended solids and low concentrations of chlorophyll-*a*, approaching 0.0 $\mu g.L^{-1}$, which can render these waters less susceptible to invasion, compared to other rivers that have lower suspended sediment concentrations.

Chlorophyll-*a* concentrations are usually low in the Paraguay River system, but *L. fortunei* evidently can use detrital organic carbon sources in addition to algae or even bacterioplankton (Calheiros, 2003; Sylvester et al., 2005) as does the zebra mussel (Baines et al., 2007).

4.1. Other factors affecting the expansion of L. fortunei

Some floodplain areas, such as the lower Cuiabá River, were also ascribed high risk based on the GARP and Maxent approaches. Although these areas are connected to the Paraguay River by frequent traffic of boats *L. fortunei* has not been recorded there after nine years in the vicinity. Six mussels were recorded in the lower Cuiabá River in the beginning of 2008, but an established population, reproducing and increasing in density, has still not been observed. In this region other factors not included in the models have to be considered, particularly oxygen depletion events, because they restrict the occurrence of *L. fortunei* by causing mortality once a year.

L. fortunei was not tolerant to dissolved oxygen conditions approaching 0.0 mg.L^{-1}, pH 5.0, $SI_{calcite}$ –4.0, and free CO_2 above 100 mg.L^{-1}, in water temperature around 30.0 °C (Oliveira et al. 2010b). During one of these events we observed that populations of *L. fortunei* were completely eliminated from a floodplain lake in the Pantanal connected to the Paraguay River. Low density in the Miranda River has been associated with dissolved oxygen depletion events also, which tend to occur during the reproductive period of *L. fortunei* (Oliveira et al. 2010b).

Seasonal water level fluctuation in the Paraguay River is about 5 m and represents another limiting factor for *L. fortunei.* It is possible that a sequence of stressful factors, such as low water levels that kill mussels by exposing them to the air, low pH during the falling and rising water phases, and depletion of dissolved oxygen during rising water might explain the lower densities of *L. fortunei* in the Paraguay River tributaries, which in turn would slow down its dispersal rate throughout the Paraguay basin. These events have been observed in the Paraguay River, connected lakes and in the lower portion of the tributaries.

In river reaches upstream of the extensive floodplains of the Pantanal these oxygen- depletion events do not occur and *L. fortunei* could potentially reach high densities in regions predicted to be of high risk of invasion, such as the Cuiabá and Miranda/Aquidauana Rivers.

Future anthropogenic alterations of these river systems may also affect their suitability for invasion by *L. fortunei*. According to Johnson et al. (2008), impoundments increase the probability of establishment of non-indigenous species, and frequently support multiple invaders. In some Paraná River reservoirs, invasive mollusks including *L. fortunei* and *Corbicula fluminea* outnumber native species (Suriani et al., 2007). Our study showed that important tributaries of the Paraguay River such as Cuiabá and Miranda/Aquidauana rivers exhibit high risk of invasion. Existing impoundments such as a large reservoir in the Manso River (tributary of Cuiabá River) and smaller ones in the Jauru, Piquiri, Sepotuba, and Correntes rivers will increase the potential of *L. fortunei* establishment, and may create more favourable habitat than has been indicated in our modeling of free-flowing river channels. The Brazilian government has plans to install several other small hydroelectric plants (<30 MW) in the UPB, and this will increase the chances of *L. fortunei* expansion and consequent problems within the reservoirs as well as in the electrical generation infrastructure.

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