Universidade Federal de Minas Gerais
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Ecologia, Conservação e Manejo de Vida Silvestre.

FATORES REGULADORES E DISTRIBUIÇÃO POTENCIAL DO MEXILHÃO DOURADO (*Limnoperna fortunei* Dunker 1857) NA BACIA DO ALTO RIO PARAGUAI E OUTROS RIOS BRASILEIROS.

Márcia Divina de Oliveira

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Orientadora: Profa. Dra. Claudia Maria Jacobi

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Sumário

Resumo

Limnoperna fortunei (Dunker, 1857), também conhecida como mexilhão dourado, foi introduzida no estuário do rio da Prata (Argentina) e rapidamente expandiu-se na bacia do rio da Prata, colonizando o rio Paraná e seu tributário, o rio Paraguai, até o Pantanal Sul-Matogrossense. Para verificar a hipótese de que *L. fortunei* se estabelecerá na maioria das águas da bacia do Alto Paraguai do Brasil, analisamos os efeitos de variáveis limnológicas no desenvolvimento de *L. fortunei* baseado nas condições do Pantanal e estabelecemos novos limites de tolerância da espécie a essas variáveis, as quais foram utilizadas na estimativa da sua distribuição potencial na bacia do Alto Rio Paraguai e outros rios brasileiros. Foram analisados parâmetros da espécie como densidade de larvas, juvenis e adultos, razão comprimento: peso da concha, crescimento da concha e condição biológica. Esses parâmetros foram relacionados com a sazonalidade das variáveis limnológicas. Utilizamos os algoritmos GARP e Maxent para modelar a distribuição potencial de *L. fortunei* e comparamos os resultados com modelos empíricos da concentração de cálcio e índice de saturação da calcita ($IS_{calcita}$). Nos modelos GARP e Maxent foram utilizadas variáveis combinadas (temperatura da água, oxigênio dissolvido, condutividade elétrica, cálcio, pH e sólidos suspensos totais) e uma única variável ($IS_{calcita}$). Corroboramos a idéia de que *L. fortunei* tem ampla tolerância a fatores ambientais e sugerimos ampliar os limites inferiores para o estabelecimento da espécie de pH > 6.0 e cálcio > 1.0 mg l^{-1} e $IS_{calcita} > -4.0$, baseado na ocorrência de *L. fortunei* no Pantanal Sul-Matogrossense. Eventos anuais de depleção de oxigênio dissolvido controlam a densidade de *L. fortunei* no Pantanal e, periodicamente, podem eliminar populações inteiras, embora não têm impedido a dispersão de *L. fortunei* nos tributários, apenas retardado. A modelagem indicou que importantes tributários do rio Paraguai, como os rios Cuiabá e Miranda/Aquidauana, exibem alto rico de invasão por *L. fortunei,* e outra grande parte exibe pelo menos risco médio. A situação é semelhante para os demais sistemas aquáticos do Brasil, os quais apresentam médio a alto risco de invasão por *L. fortunei*. Desta forma, corroboramos a idéia de que a espécie invasora *L. fortunei* se estabelecerá na maioria dos sistemas aquáticos brasileiros caso seja dada oportunidade de invasão.

Palavras-chaves: *Limnoperna fortunei*, distribuição potencial, pantanal, espécies invasoras, molusco bivalve.

Abstract

The bivalve *Limnoperna fortunei* (Dunker, 1857), also known as golden mussel, was introduced into the La Plata River estuary (South America) and quickly expanded in the La Plata basin, colonizing the Paraná River and its tributary the Paraguay River, upstream into the Pantanal wetland, Brazil. To verify the prediction that *L. fortunei* will expand to most Brazilian waters we analyzed the effects of limnological variables on the development of *L. fortunei* limits to limnological variables that were used in estimating the potential distribution of the species in the upper Paraguay basin and other Brazilian rivers. We analyzed variables such as densities of larvae, juveniles and adults, shell length:weight ratios, shell growth, and biological condition, and related them to the seasonality of chemical and physical aquatic variables. We used the GARP and Maxent algorithms to model the potential distribution of *L. fortunei* and compared the results with empirical models of calcium concentration and calcite saturation index ($SI_{calcite}$). In both GARP and Maxent models we used combined variables (water temperature, dissolved oxygen, conductance, pH, calcium, calcite saturation index and total suspended solids) and a single variable ($SI_{calcite}$). We corroborated the idea that *L. fortunei* has ample tolerance to environmental factors and propose new lower limits for the species establishment: pH > 6.0, calcium > 1.0 mg l^{-1}, and $SI_{calcite} > -4.0$, based on the occurrence of *L. fortunei* in the Pantanal wetland. Annual events of oxygen depletion control the density of *L. fortunei* in the Pantanal and can eliminate entire populations periodically, although this has not hindered the dispersal of *L. fortunei* along the tributaries, only slowed it down. Modeling indicates that important Paraguay tributaries such as the Cuiabá and Miranda/Aquidauana rivers exhibit high invasion risk, and many others show at least medium risk. The situation is similar for other Brazilian aquatic systems, the majority of them showing medium to high risk of invasion by the golden mussel. From these results we confirm the idea that the invasive species *L. fortunei* will establish itself in most Brazilian aquatic systems, given the opportunity of invasion.

Key-words: *Limnoperna fortunei*, potential distribution, Pantanal wetland, invasive species, mollusk bivalve.

Apresentação geral

O mundo globalizado tem favorecido a introdução de espécies e, consequentemente, a sua homogeneização ao longo dos continentes (Wittenberg & Cock 2001). Espécies de plantas, animais ou microorganismos introduzidos a um ecossistema do qual não fazem parte originalmente são chamadas de exóticas, e aquelas que se adaptam, propagam e exercem dominância, prejudicando processos naturais e espécies nativas, e com grande potencial para causar profundas alterações na estrutura dos ecossistemas ou mesmo danos econômicos, são chamadas espécies exóticas invasoras (Davis & Thompson 2000). Segundo o "Global Invasive Species Program" (GIS) a introdução de espécies é a segunda maior causa de perda de biodiversidade no planeta (Wittenberg & Cock 2001).

Entender os mecanismos e prever a extensão da dispersão de espécies introduzidas tem sido um desafio para muitos estudiosos nas últimas décadas (Hastings 1996; Guo 2006). Para se estabelecer numa nova área a espécie tem que ser capaz de transpor diferentes barreiras, adaptar-se para sobreviver e completar todos os estágios do ciclo reprodutivo. Dois fatores são considerados extremamente importantes na maioria dos estudos relacionados a invasões biológicas, que é a quantidade de propágulos viáveis que chegam a novas áreas e o estabelecimento da espécie, considerando a sobrevivência, condições para reprodução e a interação com outras espécies (Johnson & Carlton 1996; Williamson & Fitter 1996; Hastings 1996; Kolar & Lodge 2001; Barlow & Kean 2004; Leung et al. 2004; Hastings et al. 2005; Guo 2006, Colautti et al. 2006; Johnson et al. 2008).

O sucesso de invasão pode aumentar com o esforço de introdução, numa relação de causa e efeito, pois espécies podem ter mais sucesso porque mais propágulos ou mais tentativas podem ocorrer (Williamson & Fitter 1996; Colautti et al. 2006). Por outro lado, o sucesso de invasão está relacionado à suscetibilidade do ambiente para o estabelecimento e proliferação do invasor, como características geológicas, climatológicas e interações com a comunidade local (Colautti et al. 2006). Ambientes com maior diversidade de plantas, por exemplo, representam um importante mecanismo de defesa contra a expansão de invasores em escala local (Kennedy et al. 2002), e por outro lado, locais perturbados pelo homem mostraram maior potencial para espécies exóticas se estabelecerem segundo Johnson et al. (2008). Espécies exóticas encontram resistência da comunidade local ao seu estabelecimento, que pode ser exercida por parasitas, predadores ou competidores, o que também influencia o seu potencial de invasão (Shea & Cheson 2002).

Se a disponibilidade e qualidade do habitat afetam o estabelecimento e a abundância da espécie invasora, então seu potencial de invasão pode ser avaliado analisando as características do ambiente e as respostas da espécie ao gradiente ambiental. A modelagem da distribuição de espécies baseado no seu nicho ecológico tem sido bastante utilizada na previsão de invasão de espécies exóticas (Zalba et al. 2000; Peterson 2003; Peterson & Vieglais 2001; Shea & Cheson 2002; Drake & Bossembroek 2004; Hastings et al. 2005; Rotenberry et al. 2006).

Nos ambientes aquáticos, das inúmeras introduções associadas à navegação, tanto a marítima (Silva & Souza 2004) como a de águas interiores, somente uma pequena porção de espécies transferidas tem se estabelecido e somente uma fração desta tem se tornado praga (Moyle & Light 1996). O uso de água nos grandes navios marítimos para obter maior estabilidade, ajudar na propulsão e em manobras, a chamada "água de lastro", tem sido um eficiente meio de dispersão de organismos marinhos e de água doce. Em todo o mundo são transferidas, anualmente, cerca de 12 bilhões de toneladas de "água de lastro", que transportam aproximadamente 4.500 espécies diferentes (Bashtannyy et al. 2002).

Dentre as espécies introduzidas por meio da água de lastro, duas espécies de bivalves alcançaram os ecossistemas de água doce causando grandes danos ambientais e econômicos, tornando-se verdadeiras pragas: o mexilhão zebra *Dreissena polymorpha* (Pallas 1771), que se estabeleceu na região dos Grandes Lagos nos Estados Unidos (Claudi & Mackie 1994) e o mexilhão dourado *Limnoperna fortunei* (Dunker 1857) na América do Sul, incluindo as bacias do Alto Paraguai e Alto Paraná (Darrigran & Escurra de Drago 2000; Oliveira et al. 2006). Outra invasão de grande amplitude, embora nem sempre associada à água de lastro, é do molusco da família Corbiculidae, *Corbicula fluminea* (Muller 1774). Esta espécie é nativa dos mesmos ambientes que *L. fortunei* no Sudeste Asiático e foi introduzida também em países asiáticos, Europa e é amplamente distribuída nas Américas do Norte e do Sul (Morton 1996).

Diferentes tipos de modelos e análises estatísticas têm sido utilizados para prever a expansão de *D. polymorpha* na América do Norte e entender os mecanismos de dispersão desta espécie (Drake & Bossenbroek 2004; Ramcharan et al. 1992; Moyle & Light 1996; Shea & Cheson 2002; Bossenbroek et al. 2001; Kraft et al. 2002; Bobeldick et al. 2005; Bossenbroek et al. 2007). Johnson & Carlton (1996) atribuíram o sucesso da invasão e velocidade de expansão de *D. polymorpha* nos Estados Unidos às oportunidades de invasão, concedida pelo bisso, estrutura que permite a sua fixação em áreas produtivas como as margens dos lagos, normalmente não ocupados por bivalves nativos, constituindo nichos pouco explorados, e pela produção de larvas planctônicas (veliger), o que lhe permite ampla dispersão. Outras características compartilhadas por esta espécie, e que também se aplicam a *L. fortunei* são: crescimento rápido, alto potencial reprodutivo e ampla tolerância a variáveis ambientais (Morton 1996; Ricciardi 1998; Karatayev et al. 2007).

Morton (1975, 1982) relatou o alto potencial de *L. fortunei* se tornar um bivalve de água doce incrustante de importância igual a *D. polymorpha* e *C. fluminea,* e Ricciardi (1998) analisou a possibilidade de *L. fortunei* invadir a Europa e EUA, pois esta espécie demonstra grande potencial para expansão global através do transporte oceânico de larvas planctônicas na água de lastro. Os impactos ambientais e econômicos já são altos nos países onde *D. polymorpha* foi introduzida, e aumentará em grandes proporções com a entrada de *L. fortunei*.

O impacto dessas introduções no meio ambiente é percebido mais em longo prazo, diferentemente dos econômicos, que tendem a aparecer logo que a espécie se instala no ambiente. O impacto da introdução de moluscos bivalves exóticos tem sido mostrado através dos estudos com *D. polymorpha*, como mudanças na qualidade da água, processos biogeoquímicos e alterações da cadeia trófica (Schloesser & Nalepa 1994; O'Neill 1997; Pace et al. 1998; Strayer et al. 1999, Orlova & Panov 2004). Esta experiência tem sido utilizada para direcionar os estudos com *L. fortunei* na América do Sul, onde impactos ambientais também foram observados nos últimos anos (Darrigran et al. 1998; Mansur et al. 2003; Montalto et al. 1999; Darrigran & Escurra de Drago 2000; Boltovskoy et al. 2006, Marçal and Callil 2008).

Estudos sobre a abundância de bivalves nativos, estrutura das comunidades bentônicas e planctônicas são incipientes para se medir o impacto num ambiente tão complexo quanto a planície do Pantanal. Apesar da comunidade de peixes ser bem melhor conhecida que as demais comunidades aquáticas é difícil dimensionar em curto espaço de tempo os efeitos da troca alimentar que espécies nobres como o pacu experimentarão se alimentando em até 100% do item alimentar *Limnoperna fortunei* devido a sua abundância no ambiente (Darrigran et al. 1998; Garcia & Protogino 2005; Sylvester et al. 2005; Oliveira et al. 2006). Da mesma forma espécies invasoras podem trazer novas doenças transmitidas por vírus e bactérias que demoram a serem observadas no ambiente (Shea & Cheson 2002). Os metais acumulados pelos mexilhões, excelentes filtradores, podem ser diretamente transferidos aos peixes (Callil & Junk 1999). Mansur et al. (2003) encontraram exemplares de mexilhão dourado fixos sobre conchas e até nas partes moles de bivalves nativos como *Diplodon koseritzi* e *Leila blanivilliana* (Lea 1834) e no gastrópode do gênero *Pomacea canaliculata* (Lamarck 1822) coletados no Lago Guaíba (RS). Tais espécies nativas podem ser extintas se a colonização de *L. fortunei* for intensa e geograficamente abrangente.

Tendo em vista os inúmeros problemas relacionados à invasão de espécies exóticas, e particularmente, de bivalves como *D. polymorpha*, *C. fluminea* e *L. fortunei*, este estudo será uma contribuição ao manejo da invasão de *L. fortunei* no Brasil. Neste estudo consideramos que se *L. fortunei* vive nas condições ambientais extremas do Pantanal ela poderá viver na maioria das águas da bacia do Alto rio Paraguai e outras águas brasileiras.

A invasão de *L. fortunei* no Brasil tem por volta de 10 anos, considerada pequena tendo em vista a extensão da rede hidrográfica brasileira, o que ainda permite se estabelecer medidas de controle da invasão e, consequentemente, proteção da biodiversidade, a partir da identificação de sítios vulneráveis à invasão desta espécie nos sistemas aquáticos do Brasil.

O foco deste estudo é a invasão de *L. fortunei* na bacia do Alto rio Paraguai, mais especificamente na região do Pantanal, com o objetivo de estudar os efeitos de variáveis limnológicas sobre o desenvolvimento de *L. fortunei* e estimar sua distribuição potencial na Bacia do Alto rio Paraguai, que poderá ser utilizado em outras bacias hidrográficas.

No primeiro capítulo foram analisados os efeitos das variáveis físicas e químicas na densidade de *L. fortunei*, baseado nas condições do Pantanal. Comparações entre diferentes regiões onde a espécie ocorre, incluindo seu ambiente natural, foram feitas na tentativa de confirmar e ampliar os limites das variáveis limnológicas importantes no desenvolvimento da espécie, anteriormente propostos por Ricciardi (1998).

No segundo capítulo, avaliamos a tolerância de *L. fortunei* aos eventos de depleção de oxigênio dissolvido, eventos praticamente únicos no mundo conforme apresentado. Devido à extensa planície de inundação o Pantanal representa um lugar de condições limnológicas extremas, como estes eventos de depleção de oxigênio dissolvido, e pode representar um ambiente potencialmente impróprio para moluscos como *L. fortunei,* que apresenta mortalidades anuais durante as inundações (Oliveira et al. 2006).

No terceiro capítulo combinamos informações obtidas nos dois primeiros capítulos e realizamos previsões da distribuição potencial de *L. fortunei* na bacia do Alto Paraguai, a partir de registros de ocorrência e variáveis limnológicas, combinados em modelagem do nicho ecológico. Esta experiência foi também utilizada para uma projeção da distribuição de *L. fortunei* nos principais sistemas de rios do Brasil, onde o sucesso de estabelecimento resultará em altos custos ambientais e econômicos, como aqueles relatados para a invasão de *D. polymorpha* na América do Norte (Pimentel et al. 2005).

Bacia do Alto Paraguai e Pantanal

A bacia do Alto rio Paraguai está localizada na porção central da América do Sul, extremo norte da Bacia Platina, entre os paralelos 15°45'a 22°15' de latitude Sul e os meridianos 54°45´ a 58° de longitude Oeste. Ocupa uma área de aproximadamente 496.000 km^2, dentro dos quais está a planície do Pantanal com aproximadamente 160.000 km^2, sendo 140.000 km^2 dentro do Brasil, 15,000 km^2 na Bolívia e 5,000 km^2 no Paraguai. A região em território brasileiro possui uma área de 363.445 km² (4,6% do território nacional) e abrange porções dos estados de Mato Grosso do Sul (51,8%) e Mato Grosso (48,2%) (PCBAP 1997, Junk et al. 2006) (Figura 1).

A Região Hidrográfica do rio Paraguai apresenta importância no contexto nacional, pois inclui o Pantanal, uma das maiores extensões úmidas contínuas do planeta, considerado Patrimônio Nacional pela Constituição Federal de 1988 e Reserva da Biosfera pela UNESCO desde 2000. Junk et al. (2006) compararam sete das maiores áreas alagadas do mundo, embora o grau de conhecimento científico seja bastante diferente para cada região. O Pantanal mostra uma riqueza de espécies aquáticas comparada às outras áreas alagadas considerados por Junk et al. (2006), com 337 espécies de algas, 150 espécies de microcrustáceos, 285 espécies de rotíferos, 28 de moluscos, sendo 23 de bivalves, e 263 de peixes.

O rio Paraguai nasce na Chapada dos Parecis em Mato Grosso, é o principal rio do Pantanal, representando um canal de drenagem com 1270 km entre Cáceres e a confluência do rio Apa, abaixo de Porto Murtinho. O rio Paraguai recebe água dos tributários Jauru, Cabaçal e Sepotuba, ao norte, na margem direita. Na margem esquerda recebe os rios Cuiabá (com seus tributários rios São Lourenço e Piquiri), rios Taquari, Negro e Miranda (com seu tributário, o rio Aquidauana). Dentro do Pantanal, dos vários sistemas aquáticos, destaca-se uma série de lagos (baias) conectados ao rio Paraguai na sua margem direita. Normalmente esses lagos são rodeados de um lado por terras altas de morraria, como a Serra do Amolar, onde apresentam afloramentos rochosos.

Na região do Pantanal as principais atividades econômicas são a pecuária extensiva e o turismo, incluído o turismo de pesca e, na região de Corumbá, a extração de minério de ferro também é uma atividade economicamente importante. Atividades de agricultura no planalto representam grande impacto para o Pantanal devido ao desmatamento, que resulta na entrada de sedimentos, nutrientes e pesticidas para a planície do Pantanal. Outras atividades como a construção de reservatórios para geração de energia elétrica pode alterar o pulso de inundação no Pantanal, o qual é importante para a manutenção da diversidade biológica que se observa na região (Junk et al. 2006). A construção de reservatórios na parte alta da bacia facilita a entrada de espécies invasoras, uma vez que muitos dos reservatórios são utilizados para lazer, como a pesca esportiva. O transporte de grãos, minérios e diferentes outros produtos na hidrovia Paraguai-Paraná é responsável pela movimentação de muitas barcaças entre os cinco paises da bacia do Prata, e é considerado o principal facilitador da introdução de *L. fortunei* no rio Paraguai desde 1998, vindo do estuário do rio da Prata, na Argentina (Oliveira et al. 2006).

Área de estudo

A área principal de estudo da espécie *L. fortunei* foi o canal principal do rio Paraguai e lagos conectados, onde a espécie apresenta maior densidade (Figuras 1 e 2). Monitoramentos mensais da densidade de *L. fortunei* durante 4 anos foram realizados no rio Paraguai, próximos à cidade de Corumbá (sítios Corumbá e Porto Esperança) e no rio Miranda (Passo do Lontra). No rio Paraguai (região do Amolar) e nos lagos conectados foram realizadas amostragens apenas uma ou duas vezes ao ano. Dados limnológicos dos principais tributários do rio Paraguai (Projeto PELD/CNPq) foram utilizados para a previsão da distribuição potencial de *L. fortunei* na bacia do Alto Paraguai. Maiores detalhes sobre cada ambiente estudado são dados nos capítulos a seguir.

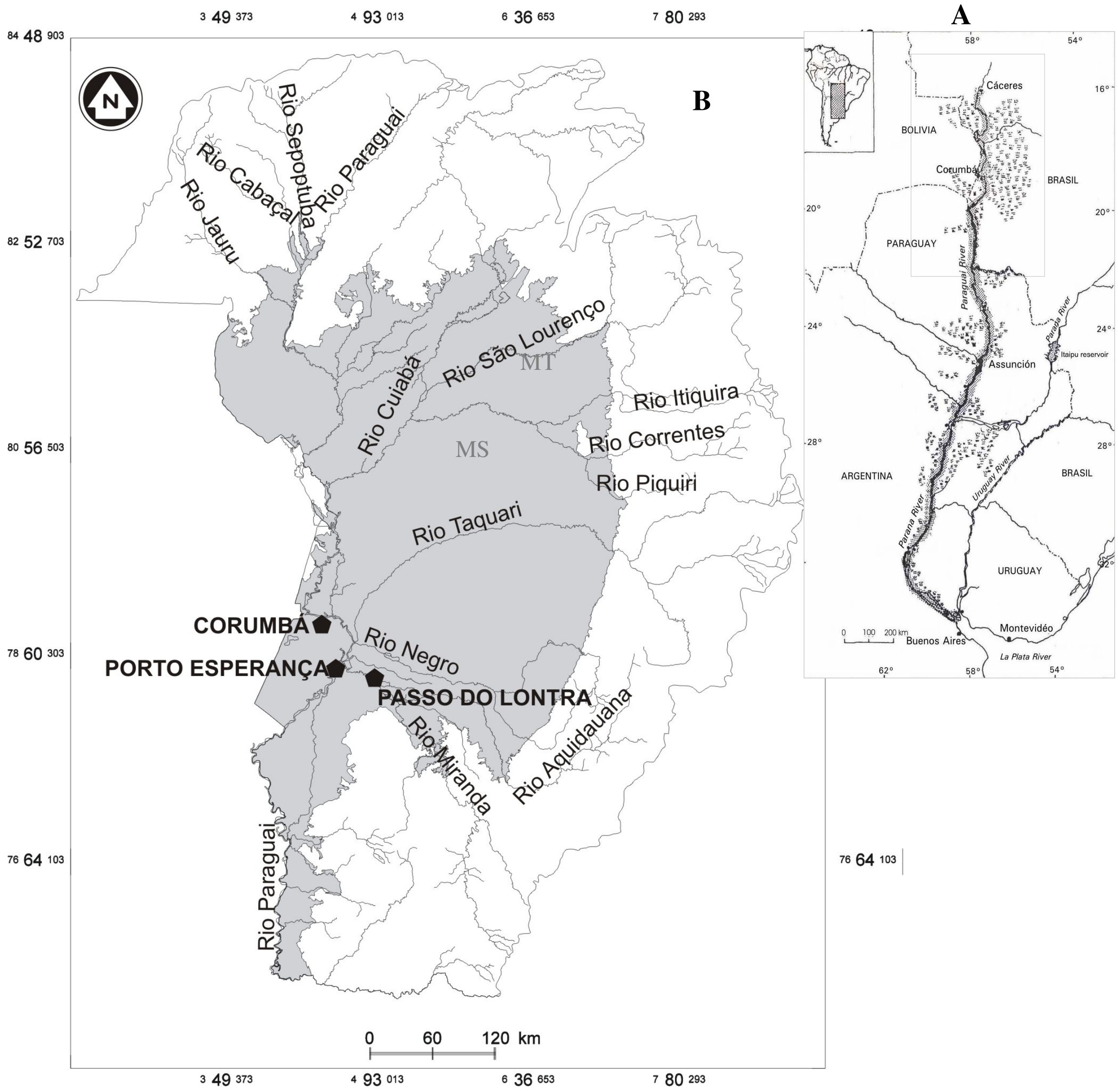

Figura 1. Localização da bacia do alto Rio Paraguai e Pantanal Sul-Matogrossense (área pontilhada) na América do Sul (A). Drenagem dos principais tributários do rio Paraguai, área do Pantanal em cinza. Círculos pretos indicam área de monitoramento mensal da densidade de *L. fortunei* nos rios Paraguai e Miranda (B).

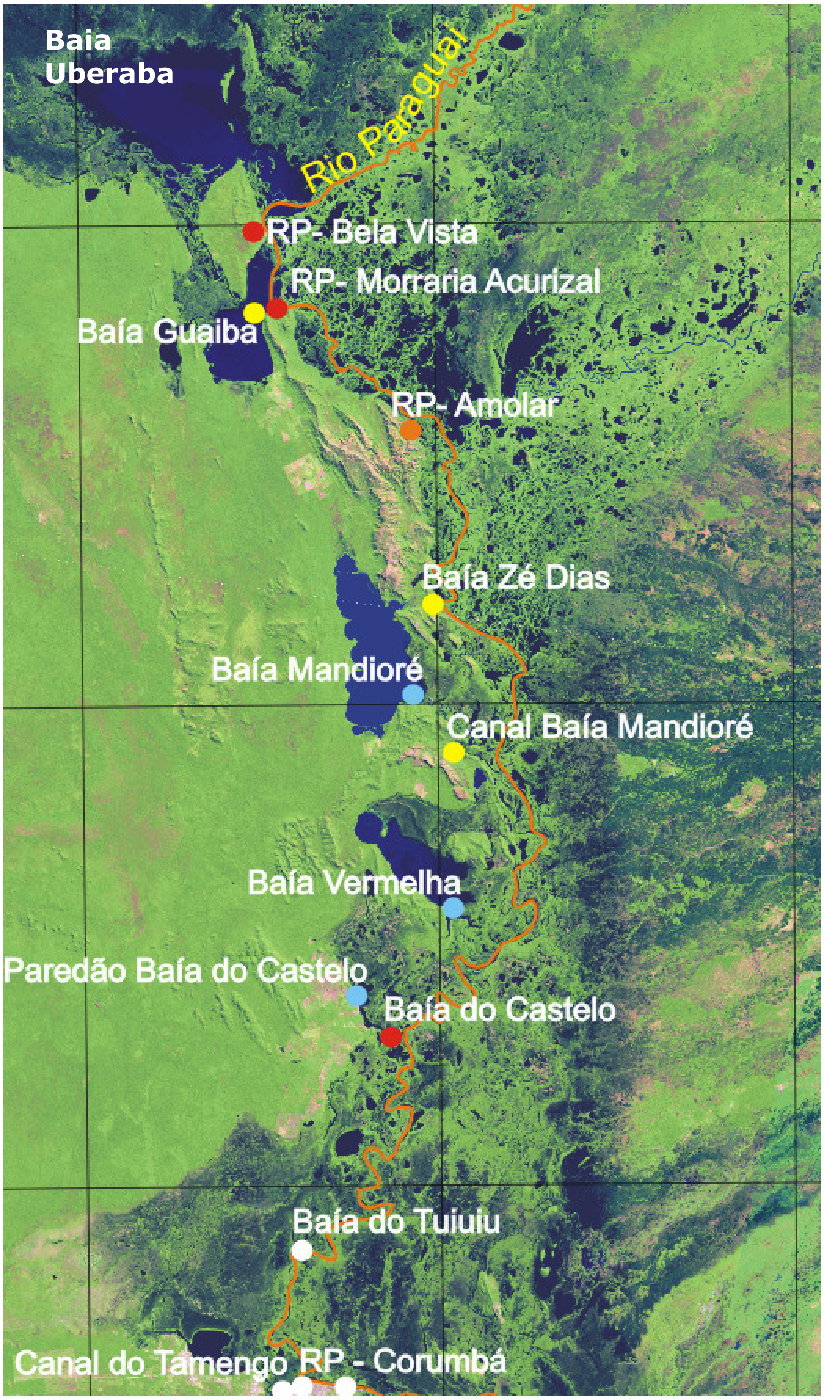

Figura 2. Rio Paraguai e lagos conectados. Círculos representam a localização das áreas de ocorrência de *L. fortunei* e amostragem (Fonte: Oliveira et al. 2004).

Capítulo 1

Factors controlling the density and distribution of the invasive golden mussel *Limnoperna fortunei* in the Pantanal wetland, a tropical floodplain.

Abstract

Limnoperna fortunei (Bivalvia, Mytilidae), a freshwater bivalve native to Southern Asia, has been an invasive species in South America since 1991, and expanded upstream reaching the Paraguay River in the vicinity of the Pantanal wetland, Brazil. In this study we examined the effects of chemical and physical variables on density of *L. fortunei*, based in the Pantanal wetland conditions. We intend to broaden the tolerance limits, and add new variables, since *L. fortunei* undergoes some extreme conditions in the Pantanal wetland and the density has been lower than in other habitats in South America. We analyzed densities of larvae and juveniles, shell length: weight ratios, shell growth, biological condition, and related them to the chemical and physical aquatic variables seasonality. In the Pantanal *L. fortunei* experiences extreme environmental conditions not found in other places where it is has been studied, such as oxygen depletion events, as well low calcium, low pH, low quality/quantity food, and high suspended solids concentration. These conditions may explain the relatively low densities, although they might not prevent the establishment of viable populations.

Introduction

The freshwater mytilid bivalve *Limnoperna fortunei* (Dunker 1857), the golden mussel, is an invasive species in South America that in many respects is comparable to the invasive dreissenid zebra mussel, *Dreissena polymorpha* (Pallas), in North America (Ricciardi 1998). *L. fortunei* was first detected in South America in 1991 in brackish waters of the La Plata River, Argentina (Pastorino et al. 1993). It was carried upstream in ensuing years, presumably aided by ship and barge traffic in the Paraguay-Paraná waterway, reaching the Pantanal wetland in Brazil (Oliveira et al. 2006). Although some controversy about the taxonomy of genus *Limnoperna* might occur, and consequently about its distribution (Lee and Morton 1983; Morton 1996), *L. fortunei* is thought to be native to two major river basins in China, the Pearl (Zhujiang) and

Yangtze (Changjiang) (Morton 1996). In the Yangtze River basin, where it has a broad distribution (Lee and Morton 1983), *L. fortunei* has been referred to as *Limnoperna lacustris* (Xie et al. 1999; Wang et al. 1999; Wang et al. 2007).

Introductions into Hong Kong, Japan and South America were mentioned by Morton (1975, 1977), Magara et al. (2001), and Darrigran et al. (1999), respectively. Morton (1987) defined *L. fortunei* as an r-selected species, adapted to the invasive colonization of a wide range of aquatic environments, with important characteristics of a successful invader such as being short-lived, capable of rapid growth, and having a high fecundity, aside from broad physiological tolerance to several factors. *L. fortunei* is recognized as a problem since it promotes large-scale fouling in water supply systems and power plants, attaining densities greater than 50,000 ind m^{-2} (Darrigran et al. 2003).

Morton (1975) suggested that *L. fortunei* has a potential for causing problems even in its native environments, being considered a pest that fouls boats and jetties in the Pearl River. No record of fouling by *Limnoperna* was found for the Yangtze River: only 1.0 to 40.0 ind m^{-2} of *L. lacustris* (*L. fortunei* according to Morton 1996) were recorded by Wang et al. (2007), in spite of its broad range of distribution in these environments and in the Pearl River.

In general, exotic species with broad distribution in their native continents should also have broad distributions in the introduced habitats if given enough time (Guo 2006). Modeling the distribution potential based on their ecological niche has been widely used to predict the spread of invasive species. To build realistic models researchers need to have good environmental data and enough scientific knowledge about the specie's biology to validate the models (Peterson and Vieglais 2001).

Success of *L. fortunei* invasion is also related to the water chemical characteristics, which will be the focus of this study. Information on abiotic limiting factors in native ranges helps to predict areas and habitats in which invasive species flourish, although the tolerance of *L. fortunei* to the chemical and physical variables that can inhibit other form of aquatic life makes difficult to identify limiting abiotic factors that would control or prevent its spread. Ricciardi (1998) suggested some limits of *L. fortunei* tolerance to the chemical and physical aquatic variables based on characteristics of invaded habitats by this species in the Asian Continent and South America. In this study we intend to broaden these limits, and add new variables, since *L. fortunei* undergoes some extreme conditions in the Pantanal wetland and the density has been lower than in other habitats in South America (Oliveira et al. 2006). The Brazilian Pantanal wetland is an ideal location to examine the abiotic factors that may limit this aquatic invasive species because of the large spatial and seasonal variation in physical and chemical water characteristics.

Methodology

Study area

The Brazilian portion of the upper Paraguay River catchment area covers about 361,666 km^2, within which the Pantanal wetland, the largest continuous floodplain area in the world, comprises about 138,183 km^2 (PCBAP 1997). The Paraguay River (mean depth 8.6 m) flows over 3,800 km from North to South collecting the waters of large tributary rivers on the left margin, including the Cuiabá, Taquari and Miranda rivers. Our study area is a portion of the Paraguay River in the middle of the Pantanal wetland and the lower Miranda River. The Miranda River basin is about 47,000 km^2 (PCBAP 1997), including upland tributaries with clear waters that originate in limestone formations of the Bodoquena hills. Deforestation by agricultural activities in the plateau areas increases sediment input along its course.

In the study area, seasonal fluctuations in river discharge and water level are unimodal, varying from 2 to 5 m. Flooding occurs as a result of river overflows, local precipitation or a combination of both processes. Flooding (rising water) in the Paraguay River occurs from February to May, followed by high water before August. The effects of the extensive floodplain on the biogeochemistry of the Paraguay River were described by Hamilton et al. (1997), and Calheiros and Hamilton (1998). The Miranda River's hydrograph responds more directly to rain events, so the flooding phase takes place earlier (between September and January at the Passo do Lontra sampling site), and floodplain inundation occurs from December to April, influenced by backwater effects of the Paraguay River.

The climate is tropical with a wet summer and dry winter. Climate in the region is tropical with marked wet and dry seasons. Mean maximum annual temperature is around 32°C in the Pantanal floodplain, ranging from 28 to 34°C. The mean minimum annual is about 20°C (PCBAP 1997).

Data collection and analysis

We examined larval and juvenile stages and limnological variables in three sites: Site 1 – Paraguay River at Porto Esperança, PR-PE (19º 36'S and 57º 26' W), Site 2 – Paraguay River near Corumbá City, PR-CR (18º59' S and 57º42' W), and Site 3 - Miranda River at Passo do Lontra, MR (19º 34' S and 57º 14.7' W). For density analysis of planktonic larvae, juveniles and adults, monthly samples were taken from January 2004 to November 2007. Limnological variables were measured every month in the Paraguay River beginning in February 2003 and from the Miranda River beginning in February 2004, both until November 2007.

For larval counts, one sample of 300 liters was taken using a Stihl motorized pump. The samples were concentrated using a plankton net of 25 µm mesh size. To determine juvenile density, we counted individuals settled onto an artificial substrate after about 30 days, defining juveniles as individuals with 0.3 to 3.0 mm shell length. We installed six units of artificial substrate in each site, attached to supports of existing bridges or docks. Each unit was a 100 cm^2 nylon net installed inside a PVC tube with a diameter of 12 cm (or PET bottle), carefully closed to prevent fish predation. The nylon net was removed each month and settled organisms were preserved in 70% ethanol for counting, as well larval samples. Because the amplitude between minimum and maximum juvenile density values was very large (0 to 700,000 ind m^{-2}) we log transformed values to achieve homogeneity of variances.

To calculate the Condition Index (CI= tissue weight x 100/ shell length) we measured shell length of mussels larger than 13.0 mm (average length of 14.2 mm in the Paraguay River and 17.2 mm in the Miranda River). Mussels were frozen before analysis. Mussel tissue was separated from shell and both were dried to constant weight at 80.0 °C, and weighed in a Sartorius precision scale 5 decimal places. Other shell weight was made in the same scale. Shell length was measured with a Sylvac caliper (mm).

Growth rate was calculated from monthly monitoring the shell length of 30 mussels ranging between 6.0-10.0 mm in shell length at the Paraguay River-PE and Miranda River sites.

For water analysis, water temperature, dissolved oxygen concentration (DO), electrical conductivity and pH were measured *in situ* with a water quality sonde (YSI). Water transparency was estimated using a Secchi disk. Total alkalinity was analyzed by the Gran titration method (Gran 1952) and free CO_2 was calculated following Kempe (1982), with modifications by Hamilton et al. (1995).

Composed water samples were taken with Van Dorn bottles at 60% of the total depth, from right and left margin and center of the channel. In the Embrapa's laboratory chlorophyll *a* was analyzed following Marker et al. (1980). Total suspended solids (TSS), inorganic (IM) and organic matter (OM) were determined by gravimetric methods, and calcium concentration was analyzed by atomic absorption, with methods based on APHA (1998). Total organic carbon (TOC) was analyzed using a Shimadzu Carbon Analyzer. Water velocity was estimated using a portable flowmeter (Flo-mate 2000, Marsh-McBirney).

Data for the river stage (water level) of the Paraguay River were obtained from daily readings carried out by the Brazilian Navy (19º02' S and 57º33' W) and by the National Water Agency (ANA), and data for the Miranda River stage were obtained from the Federal University of Mato Grosso do Sul.

To analyze how chemical and physical variables influence the seasonality of *L. fortunei* density over the course of the year and among sites we used ANCOVA, with river stage as the covariate. A simple model was used with water temperature, dissolved oxygen and river stage. Other chemical and physical variables such as pH, alkalinity, calcium, free CO_2, TSS, organic and inorganic suspended solids, chlorophyll *a* and organic carbon were related to the fluctuation of river stage.

A two-factor logistic regression was used to model the effects of temperature and dissolved oxygen on *L. fortunei* recruitment. The non-parametric Kruskal-Wallis test was used to compare the three or two sample sites, at a 5% significance level. Analyses and graphs were performed using the SYSTAT 11 software (Wilkinson 2004).To analyze how each variable was related to the seasonal fluctuation of water level and how it affected *L. fortunei* density we used non-metric Multidimensional Scaling Analysis (MDS) with Bray-Curtis similarities (Clarke and Warwick 2001). This procedure reduced chemical and physical variables (except water temperature and river stage) to 2 dimensions and tested the difference between sites and the relationships between these variables and river stage. The variables were categorized by sample site. ANCOVA was used again to test the relationship between MDS dimensions and river stage, and between dimensions and juvenile density.

Results

Reproductive activity and chemical and physical variables

Water temperature and river stage, categorized by sample sites, explained 64% (ANCOVA, $R^2= 0.557$; $p= 0.001$) of the seasonal variability in *L. fortunei* density. The highest density of juveniles and larvae was observed with mean water temperatures between 28.0 and 32.5°C, with maximum densities from November to February when temperature surpassed

30.0°C (Figure 1). Usually, between May and August settlement was not observed, or less than 10% of larvae and juveniles density was recorded.

From April to May, when the temperature dropped about 9.0°C, and after September, when water temperature rose about 8.0 °C, spawning was observed again. These results indicate that it is changes in temperature rather than absolute values (the minimum for the Pantanal wetland is 20°C, high compared to other invaded regions) that trigger the spawning in *L. fortunei*.

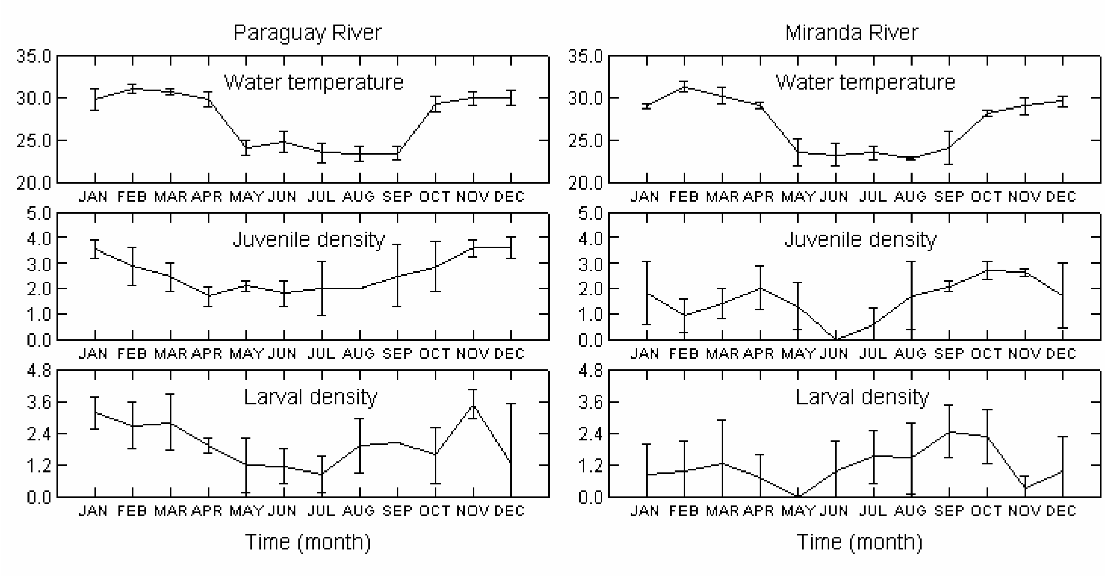

Figure 1 Seasonal variation of *L. fortunei* larvae (log ind m^{-2}) and juveniles (log ind m^{-3}) density, and water temperature (°C) in the Paraguay River at Corumbá site and Miranda River. Lines represent the mean of monthly samples from 2004 to 2007 and bar is the standard error around the means.

The Paraguay River stage, at Corumbá and Porto Esperança, varied between 1.0 and 5.0 m, and stage in the Miranda River varied from 0.0 to 3.2 m in the study period (Figure 2). Low water level caused massive die-offs of *L. fortunei* when the rocks they inhabited were exposed to air, as observed in October 2005. Density of juveniles and adults in March 2006 was lower (around 10 to 25%) than in other years monitored in this study. Although the extreme dry season had a direct effect on density, unless the mussel habitat is exposed to air, the most important effect of river stage is related to the oxygen depletion and the other chemical changes observed during the flooding.

Oxygen depletion was observed from March to April in the Paraguay River and around February in the Miranda River, during the rising to high waters phase (Figure 2). The probability of recruitment, increased with temperature and dissolved oxygen levels according to a two-factor logistic regression (*t*-ratio= -5.245, *P*= 0.000, ρ^2= 0.255, *n*= 154). The probability of recruitment was less than 10% when DO was close to 0.0 mg l^{-1} (Figure 3). These oxygen depletion events were observed every year in varying degrees and usually ceases in May, corresponding to the season when discharge is higher and the temperature drops.

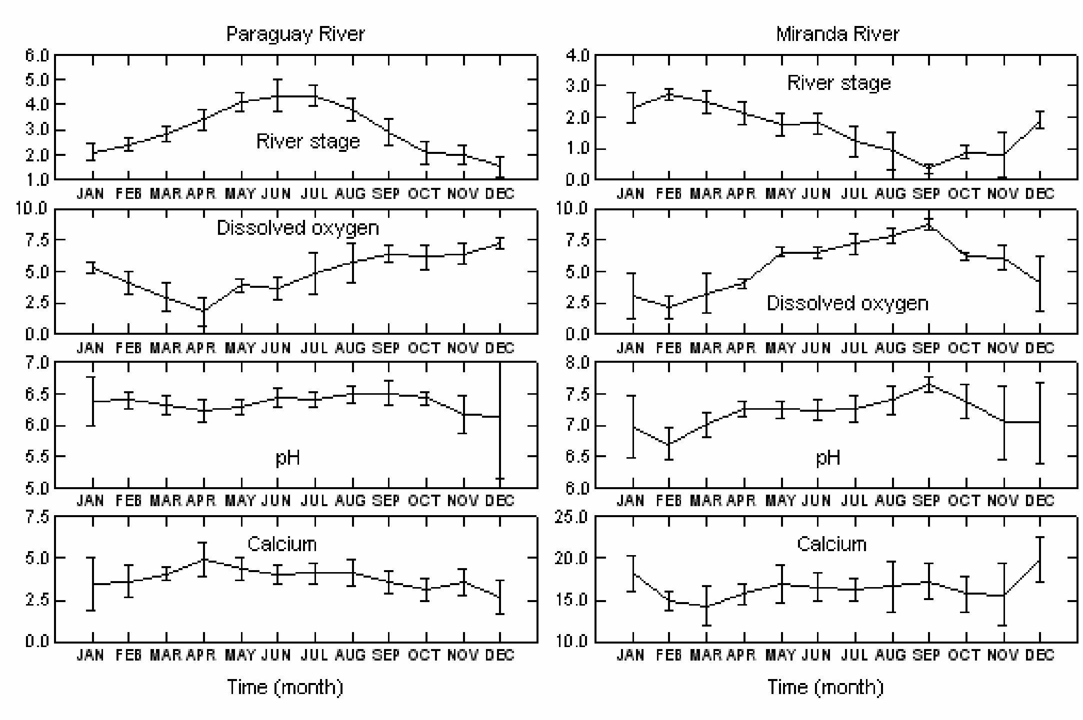

Figure 2 Seasonal variation of river stage (m), dissolved oxygen (mg l^{-1}), pH and calcium (mg l^{-1}) concentration in the Paraguay River at Corumbá site and Miranda River. Lines represent the mean of monthly samples from 2004 to 2007 and bar is the standard error around the means.

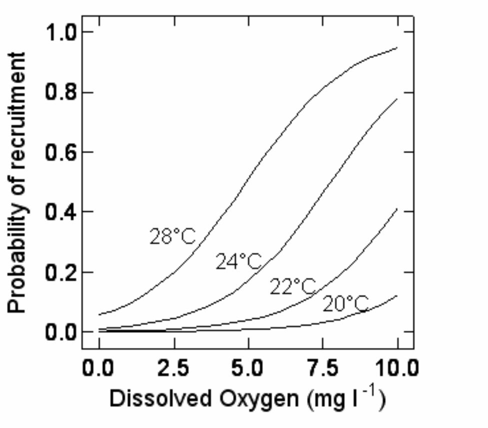

Figure 3 Trends of *L. fortunei* recruitment as a function of water temperature (°C) and dissolved O_2 (mg l^{-1}) based on a two-factor logistic regression model. Dissolved oxygen values were fixed and probability of recruitment was calculated for different water temperature (numbers along curves: 20, 22, 24, and 28.0 °C). Data for water temperature, dissolved O_2 and juvenile density were collected from February 2004 to November 2007 in the Paraguay and Miranda rivers

Other variables such as pH, alkalinity, calcium, free CO_2, TSS, organic and inorganic suspended solids, chlorophyll *a* and organic carbon were related to the fluctuation of river stage using non-metric MDS (Figures 4-7). Dimension 1 was mostly defined positively by alkalinity, pH and calcium, and negatively by TSS. Dimension 2 was defined positively by IM and TSS, and negatively by OM percentage (Figure 4).

The Paraguay River sites were separated from that of the Miranda River (MDS, R^2= 0.567, p= 0.000, stress= 0.069) with higher pH, alkalinity and calcium in the latter. Both Paraguay River sites are quite different from each other because the Paraguay River at PE receives waters from the Miranda River, influencing its chemical characteristics. Samples were distributed along a gradient between IM and OM percentage by Dimension 2 (Figure 5). At Porto Esperança the Paraguay River presents chemical characteristics intermediate between the Corumbá site and Miranda River. In some cases, such as Figure 1, we present data only from Corumbá site and Miranda River, which represent the low and high limits.

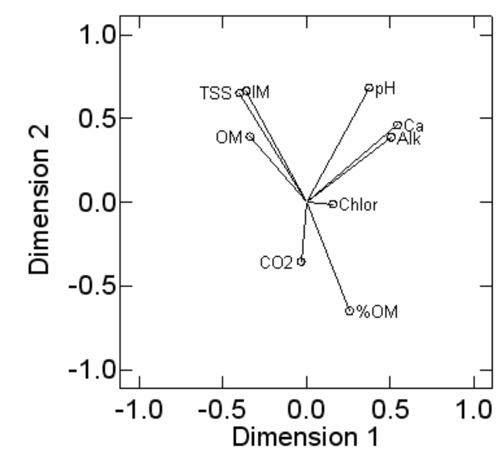

Figure 4 Factor plot resulting of MDS analysis based on standardized values of chemical variables and Bray Curtis similarities (stress= 0.069). Used variables: ALK=alkalinity, Ca=calcium, pH= pH, Chlor= Chlorophyll a, %OM= percentage of organic matter, CO2=Free CO_2, TSS= total suspended solids, IM= inorganic matter and OM= organic matter

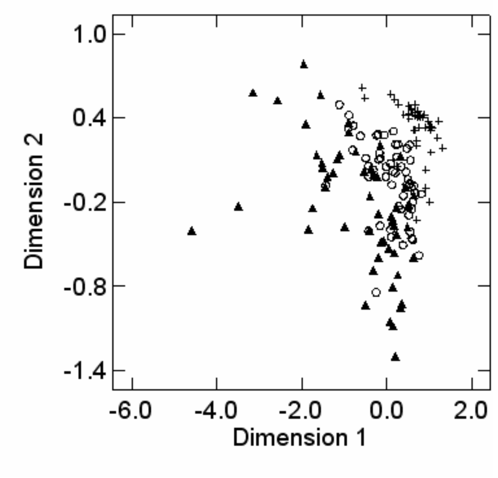

Figure 5 Two-dimensional MDS configuration for the 3 sites: PR-PE (open circle) and PR-CR (full triangle) and MR (cross).

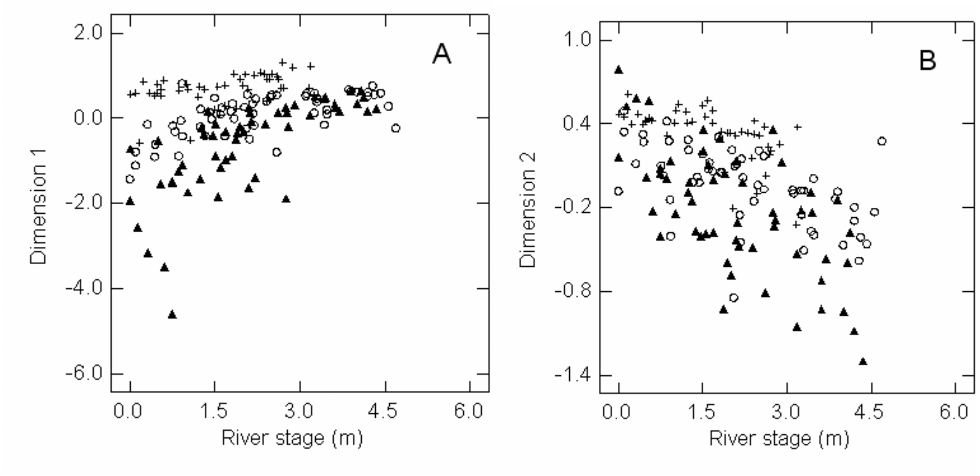

Figure 6 Linear regression model using river stage (m) against MDS dimension 1 (A) and dimension 2 (B). Symbols indicate the sample sites: PR-PE (open circle) PR-CR (full triangle) and MR (cross).

Chemical variables, more related to Dimension 1 (pH, alkalinity and calcium), increase with water level and tend to stabilize in high water (R^2= 0.136, p= 0.000) mainly in the Paraguay River (Figure 6A). Dimension 2 was negatively related to river stage in the three sites (R^2= 0.331, p= 0.000), probably because suspended solids were higher in low water (Figure 6B). Both chemical and physical variables, represented by dimensions 1 and 2, showed some influence on juvenile density (R^2= 0.049; p= 0.000; R^2= 0.459; p= 0.000, respectively) (Figure 6A and 6B). Mean pH values (Figure 2) in the Miranda River were higher than at the other sites, ranging around 7.0 to 7.5. The pH can drop during oxygen depletion events to around 6.2.

In the Paraguay River, especially at Corumbá site, these values fluctuated between 5.4 and 7.2, from 2004 to 2007, and might fall to less than 6.0 during the rising water. Some water acidification was observed during falling water mainly in the Corumbá site. Free CO_2 was higher especially during oxygen depletion events: January to March in the Miranda River and February to June in the Paraguay River. Free CO_2 varied from 1.1 to 87.0 mg l^{-1} in the Paraguay River and from 1.5 to 153.0 mg l^{-1} in the Miranda River.

Calcium concentration (Figure 2) was higher in the Miranda River, varying between 12.0 and 20.0 mg l^{-1}, compared to the Paraguay River values, between 1.0 to 6.0 and 5.0 to 9.0 mg l^{-1} at Corumbá and Porto Esperança, respectively (Kruskal-Wallis test: $H_{(2,\ N=\ 154)} = 107.2$, p= 0.000). The seasonal variation in calcium concentration over the course of the year was quite low. Lower values were observed from September to February, although in December these values were higher.

Turbidity was higher in the Miranda River (Secchi disc, 10.0 to 90.0 cm) compared to the Paraguay River (Secchi disc, 20.0 to 190.0 cm). Concentrations of TSS up to 120 mg l^{-1} were observed in the Miranda River with highest values occurring from October to December (Figure 7). Both sites in the Paraguay River contained TSS concentrations of less than 20.0 mg l^{-1}over the course of the year. Increased TSS concentrations were observed from October to February.

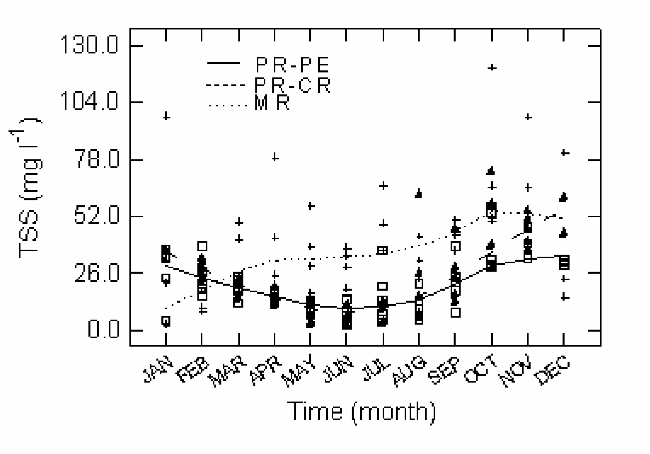

Figure 7 Seasonal variation of total suspended solids (mg l^{-1}) in the Paraguay and Miranda rivers. Symbols indicate the variability in each year, from 2003 to 2007: PR-PE, n= 56 (open circles), PR-CR, n= 54 (full triangles) and MR, n= 43 (crosses). Lines represent the mean of monthly samples.

The concentration of chlorophyll *a* at all sites was, on average, less than 1.0 µg l^{-1}. We did not find a significant difference between the Miranda River and the two Paraguay River sites for chlorophyll *a* (Kruskal-Wallis test: $H_{(2,\ N=\ 155)}= 1.74$, p= 0.4182) but we did find differences in TOC (Kruskal-Wallis test: $H_{(2,\ N=\ 120)} = 13.98$, p= 0.001), which was higher in the Miranda River (mean 1,730 ± 899 µg l^{-1}) compared to the Paraguay River at Porto Esperança (1,303 ± 980 µg l^{-1}) and Corumbá sites (1,170 ±767 µg l^{-1}). Ratios of TOC to chlorophyll *a* (Figure 8) indicate a predominance of detritus and ratios of OM to IM (Figure 9) indicate predominance of particulate inorganic material throughout the year, particularly in the Miranda River.

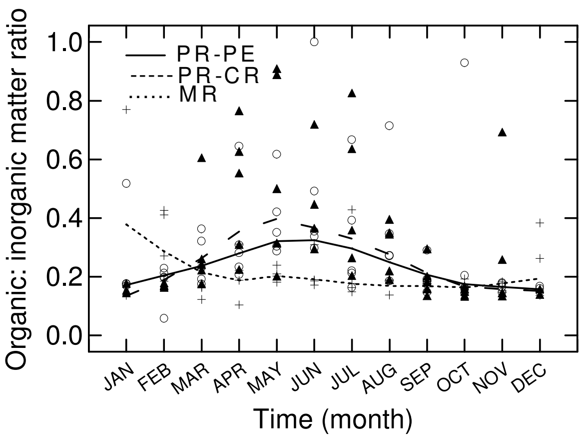

Figure 8 Organic (OM) to inorganic matter (IM) ratio (mg l^{-1}) in the Paraguay and Miranda rivers. Symbols indicate the variability in each year, from 2003 to 2007: PR-PE, n=56 (open circles), PR-CR, n= 54 (full triangles) and MR, n=43 (crosses). Lines represent the mean of monthly samples.

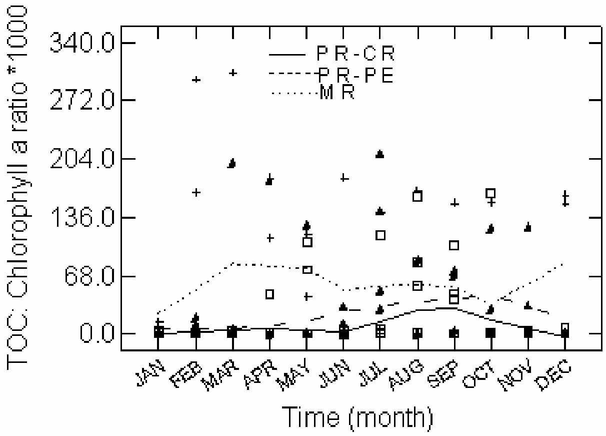

Figure 9 Total organic carbon (TOC) to Chlorophyll a (µg l^{-1}) ratio of in the Paraguay and Miranda rivers. Symbols indicate the variability in each year, from 2003 to 2007: PR-PE, n= 56 (open circles), PR-CR, n= 54 (full triangles) and MR, n= 43 (crosses). Lines represent the mean of monthly samples.

Survival and biological condition

Recruitment of *L. fortunei*, represented by planktonic larvae density and juvenile settlement, lasts around seven to eight months a year (Figure 1). In general, larval settlement was observed mainly during the spring and summer (September to April) with peaks from October to February. Larval densities were significantly different (Kruskal-Wallis test: $H_{(2, N=155)} = 8.56$; p= 0.001) among sites and juvenile density in the Paraguay River-PE was higher than the other sites (Kruskal-Wallis test: $H_{(2,\ N=\ 155)} = 64.9$, $p = 0.000$) (Table 1). Ratios of mean larval to juvenile densities suggested that survival is lower in the Miranda River compared to the Paraguay River at PE and CR (Table 1).

Table 1 Larval and juvenile density and larval to juvenile ratio. Data are mean ± SD of monthly sample from Paraguay River, at Corumbá and Porto Esperança sites, and Miranda River, from 2003 to 2007.

	Larval density		Juvenile density		larval: juvenile ratio
	N	ind m^{-3}	*N*	ind m^{-2}	
Paraguay River - CR	46	1,629 ± 4,571	46	3,149 ± 6,928	0.5
Paraguay River - PE	41	1,772 ± 4,033	46	88,523 ± 153,349	0.02
Miranda River	42	990 ± 2,292	46	377 ± 682	2.62

Substratum availability and water velocity can be important to larval settlement and survival in the Corumbá and Miranda River sites. Substratum was available at all of the study sites, in the form of hard artificial structures that can support *L. fortunei* settlement. The mean juvenile density was negatively correlated with mean water velocity (r= -0.272), and was higher in the Miranda River and lower in the Porto Esperança site (Figure 10).

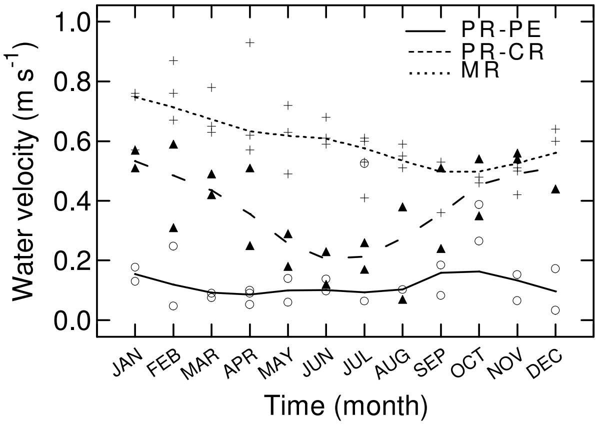

Figure 10 Water velocity (m s^{-1}) in the Paraguay and Miranda rivers. Symbols indicate the variability in each year, from 2003 to 2007: PR-PE (open circles), PR-CR (full triangles) and MR (crosses). Lines represent the mean of monthly samples.

Shell length monitoring suggested that shell growth was similar in the Miranda River and Paraguay River-PE with increments of about 0.05 mm day^{-1}, resulting in shell lengths of about 17.0 to 18.0 mm in the first year. Comparing both rivers, higher condition index values (Figure

11) and smaller shell length: shell weight (Figure12) ratios were recorded in the Miranda River (Kruskal-Wallis test: H $_{(1, N= 136)}$ = 9.6, p= 0.000 and H $_{(1, N= 136)}$ = 12.5, p= 0.000, respectively).

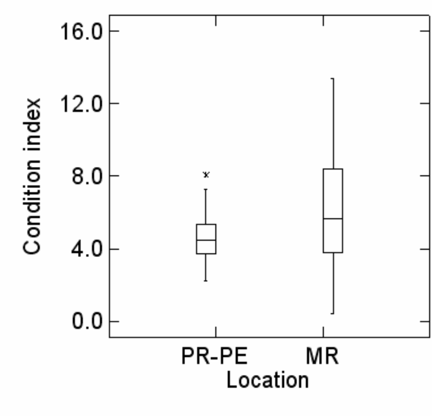

Figure 11 Condition index of *L. fortunei* collected in the Paraguay River-PE and Miranda River. In a box plot, the central horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall, with the box edges at the first and third quartiles.

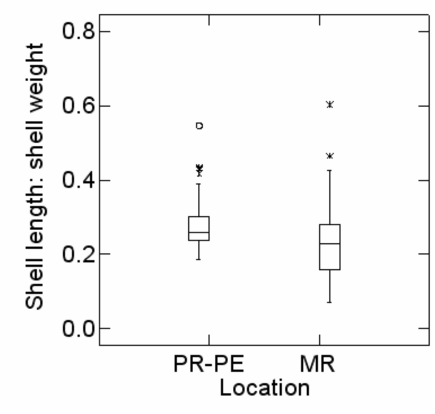

Figure 12 Relationship between shell length (mm) and shell weight (mg) from *L. fortunei* collected in the Paraguay River-PE and in the Miranda River. In a box plot, the central horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall, with the box edges at the first and third quartiles.

Discussion

Morton (1975, 1977 and 1982) demonstrated, from the first records of invasion by *L. fortunei* in Hong Kong, that this subtropical freshwater species has broad tolerance to chemical and physical variables. This tolerance makes *L. fortunei* capable of spreading to a wide range of environments in the world. The present study was developed in the Pantanal wetland where extreme conditions (i.e., oxygen depletion events, low concentrations of Ca, and low food availability) confirm and broaden the known range of tolerance of *L. fortunei*. Knowledge of how these variables affect *L. fortunei* growth and abundance in the Pantanal waters may be used to predict the spread of *L. fortunei* around the world.

Water temperature and reproductive activity

The processes of gametogenesis and spawning of *L. fortunei* have been related to changes in water temperature (Morton 1982; Darrigran et al. 2003). In the Pantanal waters *L. fortunei* did not show a clear reproductive pattern from one water body to the next and some reproductive peaks occurred irregularly, obscuring the effects of temperature on reproductive behavior. The reproductive cycle of *L. fortunei* in the Pantanal resembles that of Argentine populations, with almost continuous reproduction for about seven months of high temperatures, followed by very low reproductive activity at low temperatures (Boltovskoy and Cataldo 1999). This is quite different from results obtained by Morton (1982) in Plover Cove Reservoir, Hong Kong, where spawning occurs twice, in the summer maximum, generally triggered by rising temperature (27.0-28.0°C, June and July) and in the winter minimum, triggered by falling temperature (16.0-17.0°C, January to February).

According to Liu et al. (1979), cited by Morton (1982), *L. fortunei* in northern China, where it is widely distributed, presents only one reproductive cycle in the beginning of winter, when temperatures are between 16.0-21.0°C. This indicates that in sub-tropical to tropical regions *L. fortunei* changes its reproductive cycle markedly, for example in South America, where it reproduces mainly in the spring and summer at temperatures around 30.0°C.

In the Paraguay River *L. fortunei* is exposed to relatively high temperatures compared to its native environment in China, where the maximum annual water temperature was up to 27.8°C (Ho et al. 2003). Under laboratory conditions, Montalto and Marchese (2003) observed 40% mortality at 35.0°C after 48 hours. From our data we only may conclude that *L. fortunei* is able to maintain viable populations in water temperatures up to 32.0°C, and can tolerate extremes like 34.0°C for a short time, although Ricciardi (1998) suggested that adults of *L. fortunei* may live in temperatures up to 35.0°C. Kimura and Sekiguchi (1996) and Cataldo et al. (2005), using laboratory tests, attested that *L. fortunei* is tolerant to high temperature since the larval development was faster at temperatures around 28.0 to 30.0°C compared to 25.0°C. Our study suggests that larval development in Pantanal waters might take place at temperatures around 32.0°C. Lower temperature limits are not well known for *L. fortunei*, although this species is an invader in Japan, where minimum temperature was about 5.0-8.0°C (Magara et al. 2001).

Oxygen depletion events and other chemical variables as limiting factors

In most places where *L. fortunei* was present, DO was higher than 1.0 mg l^{-1} (Santos et al. 2005; Cataldo and Boltovskoy 1999; 2000; Villar et al. 1999; Ho et al. 2003) except for the Pantanal region, where oxygen depletion events are common. This is a peculiarity of the Pantanal wetland resulting from the contact between the Paraguay River and extensive floodplain at the beginning of the rising water phase. The oxygen depletion events, characterized by dissolved oxygen close to 0.0 mg l^{-1}, occur for a short time (usually weeks to a month or so) and are accompanied by high temperature, low pH and high free CO_2 and methane as reported also by Calheiros and Hamilton (1998) and Hamilton et al. (1995, 1997). These conditions have been observed in most years, and some years presented great fish mortality due to respiratory stress, which demonstrates the magnitude of this event. Oliveira et al. (2006) recorded substantial mortalities of mussels during oxygen depletion events in the Paraguay River.

Morton (1982) suggested that DO level affect *L. fortunei* gametogenesis and spawning. Oxygen depletion events usually occur near the end of the reproductive period in the Paraguay River and in the middle of the reproductive period in the Miranda River. In the latter case, the effect might be more severe and help to explain the lower densities in the Miranda River; we observed that the probability of recruitment was less than 10% when DO was close to 0.0 mg l^{-1}.

In general, major limnological variables investigated in this study are similar between Chinese rivers, where *L. fortunei* is native, and South American rivers considered in this study. pH is one the most important variables to predict the potential geographical range of mussels, as used by Ramcharan et al. (1992) in their study about distribution of *Dreissena polymorpha* in North America. In its natural environments *L. fortunei* lives in waters with pH above 7.0 (Xie et al. 1999; Zhang et al. 2007; Wang et al. 2007). Ricciardi (1998) suggested pH above 6.4. From laboratory tests, Montalto and Marchese (2003) recorded 80% survival in pH 5.0 after 96 hours. Maybe this duration is short to extrapolate to the natural environment. After about 5 years of invasion, *L. fortunei* has established populations in pH as low as 6.0; about 42% of monthly samples from Paraguay River at Corumbá, have pH less than 6.4. In the Paraguay River, pH can be around 5.5 during oxygen depletion conditions, but it is for a short time and usually followed by great mortality.

Karatayev et al. (2007) suggested 5.5 as the lower pH limit for *L. fortunei*, but provided no example of established populations in habitats with similar characteristics. Among the sites inhabited by *L. fortunei* in the Asian continent lower calcium concentration was observed in Sum Chum Reservoir, China, which ranges from 2.4 and 4.8 mg l^{-1} (Morton 1975). The Pearl River, the natural location of *L. fortunei* in China, carries about 32.6 mg l^{-1} of calcium (Zhang et al. 2007); Changjiang and Pearl rivers basin have the highest mineral carbonate content among the 25 largest rivers and high pH and alkalinity (Cai et al. 2008).

Our observations show that *L. fortunei* can live in water of lower ionic concentrations, as suggested by Deaton et al. (1989) that verified mussels might have byssal attachments and are filtering by end of 1 week in deionized water. The Corumbá site in the Paraguay River has the lowest calcium concentration among the known sites inhabited by *L. fortunei* (1.0 to 6.0 mg l^{-1})*;* around 17% of samples had calcium concentrations lower than 3.0 mg l^{-1}, the low tolerance limit suggested for *L. fortunei* by Ricciardi (1998). Because of the impossibility of determining the exact tolerance of *L. fortunei* to the chemical variables we need to be cautious and conservative, because this kind of data may be used to predict the spread of *L. fortunei* around the world.

Available food and suspended sediment

In waters with enough calcium such as Miranda River, other negative factors besides depletion oxygen events, such as high water velocity, low food quality and quantity, and high concentration of sediments, might explain the low larvae survival rate. The effects of a combination of high concentration of sediments (or turbidity) and low food quality and quantity have been addressed in some studies with *Dreissena polymorpha* (Madon et al. 1998; Schneider et al. 1998; Allen et al. 1999; Baines et al. 2005 and 2007). Although not addressed in studies with *L. fortunei* these variables are potentially important since most waters in the Paraguay River system have inorganic turbidity and are low in phytoplankton (Oliveira and Calheiros, unpublished data). Schneider et al. (1998) suggested that an organic:inorganic ratio lower than 0.5 be considered low food quality. This threshold is related to the inability of *D. polymorpha* to selectively ingest organic material as food quality declines. If *L. fortunei* has similar behavior, we might suppose that in the Miranda River it is not able to selectively ingest the organic fraction, since there the organic: inorganic ratio is 0.2, while in the Paraguay River food quality is better for *L. fortunei* with an organic:inorganic ratio of 0.4.

On the other hand, there is some evidence from its native environments that *L. fortunei* can live in turbid rivers with low levels of chlorophyll *a* (Xie et al. 1999; Chai et al. 2006; Zhang et al. 2007). In Guaiba Lake, Brazil, with turbidity similar to the Miranda River, *L. fortunei* attains high densities. The difference could be that chlorophyll *a* is higher in Guaiba Lake than in Pantanal environments (Santos et al. 2005). Calheiros (2003) used stable carbon isotopes to ascertain that algae and methanotrophic bacteria are the main source of carbon for *L. fortunei* in the Pantanal floodplain, and Sylvester et al. (2005) considered that algae alone cannot cover the basal requirements of adults, the remainder being accounted for by other forms of particulate organic matter. Considering the concentrations of TOC in the Paraguay and Paraná waters (Depetris and Kempe 1993), food quantity alone may not be a limiting factor to the establishment of *L. fortunei* in the Paraguay and Miranda rivers.

Condition index values in the Miranda River are slightly superior to the Paraguay River and higher compared to those obtained for mussels from Itaipu Lake, Brazil, where chlorophyll *a* is around 4.0-22.0 $\mu g\ l^{-1}$ (Silva 2006), suggesting that *L. fortunei* uses other sources of energy besides phytoplankton. Baines et al. (2007) observed that allochthonous dissolved organic matter provides an important source of food in the Hudson River, and zebra mussel takes some advantage over other organisms competing for phytoplankton.

Good condition index and greater shell length in the Miranda River suggest that low food when combined with high concentration of inorganic sediments is probably not a limiting factor for adults in the Miranda River, but can be so for larval stages, where survival is low compared to the Paraguay River. Water velocity can make larval settlement difficult and affect the larval survival. Nagaya et al. (2001) found that fluid velocity around 1.0 $m\ s^{-1}$ was critical to attachment of juvenile of *L. fortunei* and the water velocity needed to detach mussels increases with shell size. High densities of *L. fortunei* were recorded in water velocities of 0.1-0.8 $m\ s^{-1}$ in the Paraná de Las Palmas River (Cataldo and Boltovskoy 1999), but we need to consider the differences in the surface properties of the substrate. Water velocities from 0.4-1.1 $m\ s^{-1}$ in the Miranda River seem to have hindered settlement onto artificial substrata in the present study, although more information is necessary to make conclusions about this effect.

Time of invasion and reintroduction affecting density

The time of introduction seems not to be important in determining densities because, considering the lifespan of about 2-3 years (Morton 1982), 5-10 years would be enough time for population stabilization as postulated by Darrigran et al. (2003). Instead, propagule pressure might be one important factor to explain the success of invasion in the Paraguay River at Porto Esperança site compared to the other two sites, as suggested by Barney and Whitlow (2008) and Colautti et al. (2006). Reintroduction is expected at Porto Esperança, where vessels from the Paraná River, most likely with incrustations of *L. fortunei*, dock for more than one day.

One example of lack of successful persistence of a new population of *L. fortunei* is in the northern Pantanal, where its presence was confirmed once but it disappeared in subsequent years

(C T Callil personal communication). At that site less opportunity for re-colonization can be attributed to the low vessel traffic, in addition to the oxygen-depleted episodes observed in some years. In the Miranda River, although the mussels have spread upstream presumably by boat traffic, the lower amount of traffic and smaller size of boats might help explain their lower mussel density. Nevertheless, in spite of the extreme conditions *L. fortunei* has advanced into the Paraguay River, tributaries and floodplain, suggesting that it can survive, albeit in lower densities.

Another factor affecting colonization success is the nature of the environment. Altered environments such as impoundments facilitate the introduction and establishment of invasive species Johnson et al. (2008). This factor might increase the colonization success of *L. fortunei* in the Alto Paraguay River basin, where, although still maintaining its hydrological characteristics, the current energy generation reservoirs and those planned for a near future will accelerate its spread.

Capítulo 2

Importance of oxygen depletion events in controlling the invasive golden mussel (*Limnoperna fortunei* Dunker, 1857) in rivers and wetlands of the Brazilian Pantanal

Abstract

Over the past 10 years the golden mussel (*Limnoperna fortunei*), an exotic species native to eastern Asia, has become widespread and abundant in the Pantanal wetland. Oxygen concentrations are often low in rivers and floodplain waters of the Pantanal and oxygen depletion events can last for several weeks during the rising water phase. In spite of the evidence of *L. fortunei* mortality during oxygen depletion events, its tolerance to hypoxic and anoxic conditions is poorly understood, in part because oxygen depletion is accompanied by a complex combination of changing variables. In this study we analyzed the effects of oxygen depletion events on *L. fortunei* density and its tolerance to the environmental changes during these events. We also explore the main factors, in addition to low oxygen availability, that may affect *L. fortunei* during oxygen depletion events, such as acidification water and shell decalcification, and organic compounds effects. We analyzed interannual variation in densities of adults and juveniles corresponding to varying oxygen availability, and tested the tolerance of *L. fortunei* to oxygen depletion events in a floodplain lake (Castelo Lake) and in the laboratory. We monitored limnological variables including water temperature, dissolved oxygen, pH, free CO_2 and calcite saturation daily during the season of oxygen depletion events. *L. fortunei* could not tolerate the oxygen depletion events, which involve a combination of low dissolved O_2, low pH, and high free CO_2. Mussels died after 5 days immersed in oxygen depletion events water at Castelo Lake, and a population established there in 2005 was extirpated in 2006 after an oxygen depletion event. Laboratory tests confirmed that mussels died more quickly in water from the oxygen depletion event. Annual oxygen depletion events must control the mussel populations in the Pantanal, maintaining low densities and periodically extirpating them from some habitats.

Introduction

Over the past 10 years the golden mussel (*Limnoperna fortunei*), an exotic species native to eastern Asia, has become widespread and abundant in the Paraguay-Paraná river system, which extends through subtropical and tropical climates in five South American countries (Oliveira et al. 2006). Although the spatial prediction of species distributions has been recognized as important to invasive species management and conservation planning, incomplete understanding of environmental requirements of these species makes it difficult to predict how much further invasive species can spread.

L. fortunei is found at high densities in most places where it has been introduced throughout the Paraguay-Paraná river system, mainly in Argentina and southern Brazil. In the Pantanal wetland, however, densities remain low over 10 years after its introduction (Oliveira et al. 2006). Low concentrations of calcium and low pH may limit the density or restrict the establishment of bivalves since in most mollusks shell formation is related to the available calcium in the environment (Mackie and Flippance 1983; Ramcharan et al. 1992; Checa et al. 2007). Calcium concentration and pH have been used to predict the distribution and density of an invasive mussel species in North America, *Dreissena polymorpha* (Ramcharan et al. 1992), because occurrence and density were highly correlated with these aspects of water chemistry.

There is less information about the water chemistry of habitats colonized by *L. fortunei*, and most reports lack information on seasonal variability. Studies in Southeast China, where *L. fortunei* is native (Morton 1982; Magara et al. 2001; Goto 2002), and in Argentina, where it is an invader, have examined population dynamics and temperature effects on reproductive activity (Cataldo and Boltovskoy 1999 and 2000; Darrigran et al. 2003), so some data exist about habitat suitability including the minimum temperature requirements.

In spite of broad similarities between native environments (Southeast China) and those where it has been introduced (South America), as verified by Oliveira et al. (in review), some South American habitats might be more prone to seasonal events of hypoxia or even anoxia that could control the density of *L. fortunei*, and might restrict its establishment.

Hypoxic and anoxic events, defined as low oxygen (< 2.0 mg l^{-1}) and essentially no oxygen, respectively, are commonly observed in the estuaries of large rivers such as the Mississippi, Yangtze, Pearl and Rhône rivers (Rabouille et al. 2008) and effects on aquatic communities have been noted by Rowe (2001) and Rabalais et al. (2002) in the lower Mississippi Delta.

In spite of the evidence of mussel mortalities during oxygen depletion events in the Pantanal wetland (Oliveira et al. 2006), the tolerance of *L. fortunei* to hypoxia and anoxia is not well understood, in part because these events entail a complex combination of changes in chemical variables, in addition to depletion of dissolved oxygen, such as acidification due to increases in dissolved free carbon dioxide (Oliveira et al. 2006). Morton (1977) also observed lower densities of *L. fortunei* under low concentrations of dissolved oxygen.

Gazeau et al. (2007) demonstrated the dependence of mussel calcification rates on pCO_2 and carbonate ion (CO_3^{-2}) concentrations in ocean waters. Acidification of marine waters by increasing atmospheric CO_2 and consequent effects on calcification processes have been the subject of much recent research (Kleypas et al. 2006). Exposure to elevated CO_2 can affect physiology as well as calcification rate in many other benthic organisms, but the molecular and physiological mechanisms that control the response of mollusks to changes in the dissolved CO_2 system are poorly understood.

In this study we analyzed the effects of oxygen depletion events on *L. fortunei* density and its tolerance to the environmental changes during these events. We also explore the main factors, in addition to low oxygen availability, that may affect *L. fortunei* during oxygen depletion events, such as acidification water and shell decalcification, and organic compounds effects.

Methodology

Study area

The Pantanal wetland is one of the largest freshwater wetlands in the world, located in the upper Paraguay basin (UPB). Climate in the region is tropical with marked wet and dry seasons. The maximum daily temperature averages around 32°C in the Pantanal floodplain, with monthly means ranging from 28 to 34°C. The mean annual minimum daily temperature is about 20°C (PCBAP 1997). The Brazilian portion of the upper Paraguay River catchment covers 363,445 km^2, and the Pantanal floodplain extends over about 140,000 km^2 (PCBAP 1997). The Paraguay River, more than 3,800 km long, flows from north to south along the western side of the Pantanal. It receives water from large tributary rivers on the left margin, such as the Cuiabá, Taquari and Miranda. Flooding occurs as a result of river overflows, local precipitation or a combination of both processes, and can be delayed by over three months in relation to rainfall in the basin. In general, flooding begins after September in the North and water accumulates in the floodplain due the very low slopes (PCBAP 1997).

The extensive contact between rivers and floodplains of the Pantanal produces marked changes in water chemistry, particularly during the rising water phase. Visual and chemical changes in the water are locally called ‘decoada or dequada’ as explained by Calheiros and Hamilton (1998) and Calheiros et al. (2000). In this study we used the term “oxygen depletion events” to refer to periods of several weeks at rising water in which water on the floodplains and returning from the floodplains to the rivers contains little or no oxygen (Hamilton et al. 1995, 1997).

A detailed description of effects of the extensive floodplains on the biogeochemistry of the Paraguay River at its outflow from the region, including analysis of oxygen depletion events, appears in Hamilton et al. (1995 and 1997), and Calheiros and Hamilton (1998). Besides changes in color reflecting higher concentrations of dissolved organic carbon, changes in dissolved O_2 are the main chemical characteristic to identify the oxygen depletion events (Hamilton et al. 1997). Oxygen depletion events are mainly observed along the course of the Paraguay River, including connected lakes and floodplain waters and lower sections of main tributaries. In the more southern tributaries such as the Miranda River, flooding occurs between December and March, earlier than in the Paraguay River (Hamilton et al. 1995).

On the right margin of the Paraguay River there is a series of large floodplain lakes, connected to the river and partially surrounded by higher land (Hamilton et al. 1995). Most connected lakes along the Paraguay River exchange water with the river and can contribute to changes in river chemistry particularly during rising and falling water levels. Castelo Lake is a typical example of these lakes, and is surrounded by an extensive vegetated lowland area which is seasonally inundated. During inundation, emergent aquatic and semi-aquatic plants colonize much of this area; the most abundant species include *Scirpus cubensis*, *Eichhornia azurea*, *E. crassipes*, *Oryza* spp., and *Polygonum* sp. (Calheiros and Hamilton 1998). Much of this biomass dies during the dry season, and in the subsequent inundation the dead vegetation will be leached and decomposed, influencing the chemistry of the lake as well as the Paraguay River.

Data collection and analysis

Observations of oxygen depletion events in the Paraguay River and floodplain

An oxygen depletion event was considered underway when dissolved O_2 was below 2.0 mg l^{-1}, based on results from Calheiros and Hamilton (1998) and Hamilton et al (1997). To observe the oxygen depletion events in the Pantanal floodplain we monitored limnological characteristics from 2003 to 2007 at six sites: the Paraguay River at Corumbá city (PR-CR; monthly measurements), four lakes connected to the Paraguay River (Gaiva, Mandioré, Zé Dias, and Castelo; annually measurements), and one site in the Miranda River near its confluence with the Paraguay River (MR; monthly measurements).

Limnological variables such as water temperature dissolved O_2, pH and conductance were measured daily over the course of oxygen depletion events in 2006 and 2007 in the Miranda River and Castelo Lake to investigate shorter-term dynamics. These variables were monthly monitored in the Paraguay and Miranda rivers, and annually in the connected lakes.

Total alkalinity was analyzed by the Gran titration method (Cantrell et al. 1990) and free CO_2 was calculated from pH, alkalinity, major ion chemistry, and temperature according to Kempe (1982), with modifications by Hamilton et al. (1995). Dissolved Ca^{+}, Mg^{2+}, Mn^{2+}, and Fe^{2+} were analyzed by atomic absorption, Na^{+} and K^{+} by flame atomic absorption spectrophotometry, and SO_4^{-2} by the turbidimetric method (APHA 1998). Dissolved organic carbon (DOC) was analyzed by high-temperature Pt-catalyzed combustion according to Benner and Strom (1993), and dissolved inorganic carbon (DIC) by stripping and infrared gas analysis according to Hellings et al. (1999). Total N and P were analyzed by colorimetric methods after persulfate digestion (Valderrama 1981), and chlorophyll *a* was measured spectrophotometrically according to Marker et al. (1981).

Calcite and aragonite are the most common $CaCO_3$ polymorphs in mollusk shells and the formation of these minerals is related to the Mg: Ca molar ratio; molar ratio of Mg: Ca < 2 is more favorable to calcite formation (Checa et al. 2007; Stabel 1986; Takeuchi et al. 2008). Molar ratios of dissolved Mg:Ca in the Paraguay and Miranda rivers were around 0.2, so we used the calcite saturation index ($SI_{calcite}$) to understand the thermodynamic propensity for calcification in these waters, which are characteristically undersaturated with respect to calcite but vary considerably in the degree of undersaturation. $SI_{calcite}$ was calculated using water temperature, pH, conductance, total alkalinity, and dissolved calcium based on APHA (2005) after confirming that results were similar to calculations using full major ion chemistry following Kempe (1982) and Hamilton et al. (1995). The $SI_{calcite}$ is 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.

Water level data for the Paraguay River at Ladário City were obtained from the Brazilian Navy, and data for the Miranda River at Passo do Lontra were obtained from the Federal University of Mato Grosso do Sul.

The effect of oxygen depletion events on juvenile and adult densities

Densities of juvenile mussels, defined as individuals from 0.3 to 3.0 mm shell length (Cataldo et al. 2005; Santos et al. 2005), were monitored on artificial substrata. Each substratum was composed of a 100 cm^2 Nylon net installed inside a PVC tube with a diameter of 12 cm (or a recycled PET bottle), carefully closed to prevent fish predation. Average annual density of juveniles was estimated during the spawning period before the oxygen depletion event commenced but after the minimum water level: January to March in the Paraguay River and October to December in the Miranda River.

Density of adults was estimated by counting all individuals longer than 5 mm on rocks with known area, annually, and was expressed as ind m^{-2}. One sampling area of about 12 m^2 (2 x 6 m^2, 12 quadrangle of 1 x 1 m^2) was established on the edge of each water body and three rocks (0.03 to 0.08 m^2) were collected from the bottom in each of five quadrangles randomly located within the sampling area (Meese and Tomich 1992).

A two-factor logistic regression was used to model the effects of dissolved oxygen on *L. fortunei* recruitment. ANOVA repeated measures were used to compare the different treatments in the laboratory experiments at a 5% significance level. Analyses and graphs were performed using the SYSTAT 11 software (Wilkinson 2004).

Mortality of *L. fortunei* in oxygen depletion events

A. Field tests

To test the tolerance of *L. fortunei* to oxygen depletion events, two *in situ* experiments were conducted in Castelo Lake, in 2006 and 2007, during oxygen depletion events. In the first experiment, four local stones (between 0.02 to 0.10 m^2) with incrustations of *L. fortunei* were taken from the bottom and maintained in suspension in the water column of the lake, at about 1.0

m from the bottom, from February to May 2006. The number of mussels longer than 5 mm shell length was estimated twice before, and after an oxygen depletion event.

For the second experiment (March 2007), we collected mussels of about 7.0-10.0 mm shell length in the Paraguay River, and acclimatized them in an outdoor tank at the laboratory for one week. Thereafter, 50 of the mussels were placed in a small bag with 1.0 mm mesh size and immersed in Castelo Lake at depths of ~0.5 and 1.0 m from the surface during the oxygen depletion event. As a control, another 50 mussels were maintained in the tank water at ambient temperature (about 26.0°C) along the edge of Castelo Lake. We did not use aeration but changed the control water once a day. The condition of the mussels was verified every 3 hours, together with measurements of water temperature, dissolved O_2, and pH. Calcium and alkalinity samples were taken at the beginning and end of the experiment. The experiment was continued until total mortality of the mussels in the lake water was attained. Survival was estimated considering the mortality rate by exposure time.

To test the survival of *L. fortunei* in Castelo Lake outside the oxygen depletion event we monitored, monthly, the shell length of about 30 mussels (longer than 6 mm) immersed in the Castelo Lake, from September 2006 to March 2007. Mussels were placed in a small bag of 1.0-2.0 mm mesh size to avoid fish predation. The experiment stopped because mussels died during the 2007 oxygen depletion event.

B. Laboratory tests

Laboratory experiments took place in April 2007 and May 2008. In the first experiment (2007) we tested the survival of *L. fortunei* in water from oxygen depletion events under different water temperatures (26.0± 1, 32.0± 1, 34.0± 1 and 36.0± 1° C). Experimental results for 32.0 and 34.0°C were similar and therefore were combined for graphical analysis, and 33.0°C. Ten mussels from the Paraguay River, with shell lengths between 10.0 and 15.0 mm, were rinsed and placed in a 600-mL beaker with oxygen depletion events water from Castelo Lake. A treatment with control water from the laboratory tank was maintained for each temperature. Three replicates of each treatment were maintained at each temperature during 60 hours, the time over which we had observed total mortality in the *in situ* experiment(March 2007). Survival was estimated considering the mortality rate by exposure time.

In the second experiment (May, 2008), a “DOC-free” treatment was conduced to test the effects of organic compounds in mussel’s mortality. Mussels were incubated in water collected from oxygen depletion event and in the same water in which dissolved organic compounds had been removed by passing it through a Barnstead activated charcoal column. Three replicates were used for each experiment and 10 mussels in each 600-mL beaker. $CaCO_3$ was used to raise pH around 6.5 in 'DOC-free'water. To provide food for the mussels, 1.0 ml of water rich in algae, obtained from the laboratory tank and concentrated in 36 µm plankton net, was added to each beaker of DOC-free water.

Water temperature, dissolved O_2 and pH were recorded every 3 hours until 264 hours. The experiment was conducted at a temperature of 32.0°C. *L. fortunei* is sensitive to low dissolved oxygen below ~0.5 mg l^{-1} (M.D. Oliveira unpublished data), so we considered only samples where oxygen was above 0.5 mg l^{-1}. In all experiments mussels were checked for survival by examining open shells and looking for movement.

Decalcification assays

Shell decalcification, as evidenced by thinning, has been observed during oxygen depletion events. To test the effect of acidic water on calcification during oxygen depletion events, a simplified design based on Gazeau et al. (2007) was used. They calculated net calcification rates using an alkalinity anomaly technique, based on the reaction wherein the precipitation of 1 mole of $CaCO_3$ consumes 2 moles of HCO_3^- (and yields 1 mole of CO_2). In our case, we expected to see net decalcification by the reverse reaction, resulting in net increases in dissolved Ca^{2+} and carbonate alkalinity: $CaCO_3 + CO_2 \rightarrow Ca^{2+} + 2HCO_3^-$.

Several tests were performed to develop a closed system that avoided air-water gas exchange, and to determine the optimum duration of incubation and quantity of mussels. We conducted incubations (3 replicates) without mussels also to assure that changes in water chemistry were due to the presence of mussels. Experiments were conducted using 50 g wet weight of live mussels that had been cleaned and acclimatized for one week in the laboratory tank. Mussels were placed in a 2-liter closed system and incubated for 2 hours. To avoid external gas exchange we installed a plastic bag inside the closed-system and expanded it as water samples were withdrawn by injecting air with an aquarium air pump. Sensors measured water temperature, dissolved O_2 and pH in the closed system at 15 min intervals. Samples for total alkalinity and dissolved calcium (filtered through GF/F 0.45 µm filters) were taken every 15-30 minutes. Water in the closed system was well mixed before samples were taken. Experiments were conducted using water collected during the oxygen depletion events and during normal times from Castelo Lake.

The net decalcification rate was calculated using linear regression of alkalinity against time of incubation. Consumption of O_2, production of CO_2, and changes in calcium concentration were similarly analyzed. Non-significant slopes were considered to be rates of zero in the compilation of results. The net increase in concentrations of total alkalinity or Ca^{2+} was converted to the equivalent dissolution of $CaCO_3$ assuming 2 moles of alkalinity and one mole of Ca^{2+} were produced for every mole of $CaCO_3$ that dissolved, and results were then expressed as Ca released from $CaCO_3$ per unit wet weight of mussels per hour. The reactive $CaCO_3$ is presumed to be shell material because previous tests conducted across a range of conditions revealed no carbonate minerals in suspended or epiphytic particulate matter in waters of the Paraguay River within the Pantanal (Hamilton et al. 1995).

Results

Observations of oxygen depletion events in the Paraguay River and floodplain

During much of the year, dissolved O_2 concentrations in the Paraguay and Miranda rivers were below atmospheric equilibrium but above the hypoxic level. Oxygen depletion events, defined as oxygen concentrations falling below 2.0 mg l^{-1}, were recorded during the rising water phase in the Paraguay River (March to May) and in the rising to high water phases in the Miranda River (December to March) over the course of the study. Oxygen depletion events in the Paraguay and Miranda rivers are shown in the Figure 1.

In the Paraguay River the oxygen depletion events lasted longer in 2006 and 2007, and were shorter, restricted to April, in 2004 and 2005. The minimum Paraguay River stage during the 2004 low water phase was about 1.5 m and water level in the following inundation phase (2005) was low compared to subsequent years. With less contact between river and floodplain, depletion of O_2 was weak during the 2005 rising water phase.

Other chemical changes were not always proportionate to the O_2 depletion. Although the minimum O_2 concentration in the Paraguay River was only about 1.8 mg l^{-1}, marked acidification (pH ranging from 5.4 to 6.0) and high free CO_2 (around 87.3 mg l^{-1}) were recorded between November 2004 and April 2005, as well as in November 2005. In 2006 and 2007, dissolved O_2 was low (close to 0.0 mg l^{-1}) but pH was slightly higher (6.2). Other characteristics such as $SI_{calcite}$ and free CO_2 showed greater changes in 2004 to 2005, coincident with pH changes; $SI_{calcite}$ oscillated around -3.0, and reached -4.0 in the 2005 oxygen depletion event.

Free CO_2 was around 10.0 mg l^{-1} over the course of the year, then reached ~80.0 mg l^{-1} during the 2005 oxygen depletion event.

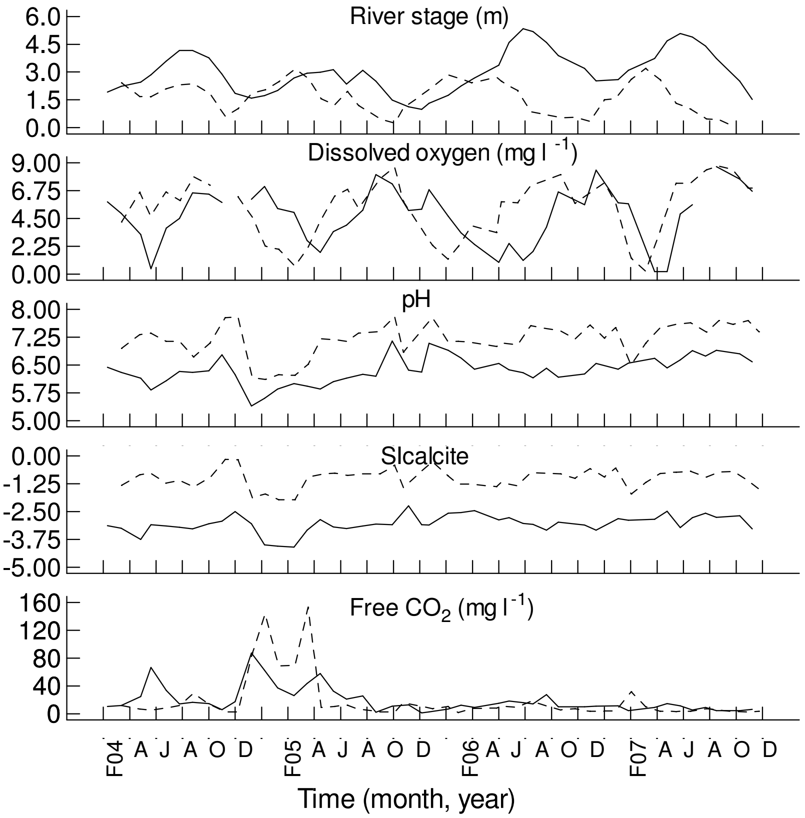

Figure 1. Seasonal variation in river stage (m), dissolved O_2 (mg l^{-1}), pH, $SI_{calcite}$ and free CO_2 (mg l^{-1}). Samples were taken from 2004 to 2007 in the Paraguay (solid line) and Miranda River (dotted line).

Dissolved O_2 variation in the Miranda River was similar to that in the Paraguay River, but pH and calcite saturation were higher in the Miranda River, which carries water of higher alkalinity. In the Miranda River the oxygen depletion event was more intense in 2005 and 2007. Dissolved O_2 dropped from 8.0 to 0.0 mg l^{-1}, pH fell as low as 6.2, and the maximum free CO_2, recorded in 2005, was 153 mg l^{-1}. The $SI_{calcite}$, which was often about -1.0 in normal times, fell to close to -2.0 (Figure 1).

The oxygen depletion events in 2004 and 2006 were weak, with dissolved O_2 never below 1.0 mg l^{-1}, and pH remaining around 7.0. The oxygen depletion events in the Miranda River were more intense in 2007, when minimum dissolved O_2 was close to 0.0 mg l^{-1} for about two months and the minimum pH was about 6.2 (Figure 2). Calcite was undersaturated in both periods, but more so in 2007 ($SI_{calcite}$ often below -1.5) than 2006 ($SI_{calcite}$ often above -1.5).

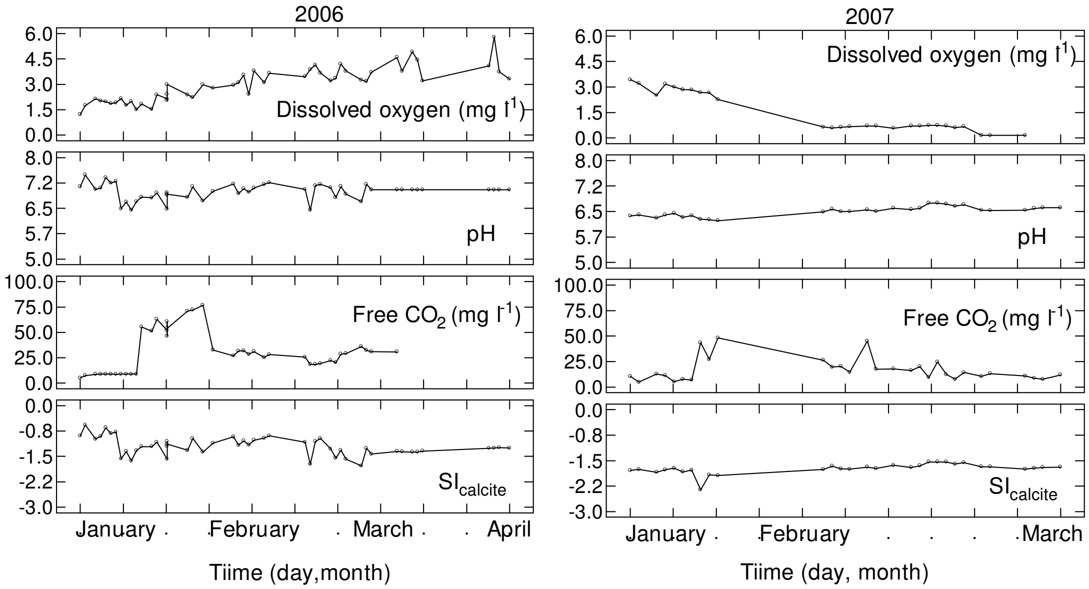

Figure 2. Short-term variation of dissolved O_2 (mg l^{-1}), pH, free CO_2 (mg l^{-1}) and $SI_{calcite}$ during oxygen depletion events in the Miranda River, between January and April 2006 (left graph) and January and March 2007 (right graph).

Oxygen depletion events lasted for about a week to a month, and occurred once a year. Low dissolved O_2 (<1.0 mg l^{-1}), low pH (<6.0) and low calcium (<3.0 mg l^{-1}) were more frequent and lasted longer in the Paraguay River than in the Miranda River (Figure 3). Significant differences for several variables were found when comparing an oxygen depletion event with normal conditions in the Paraguay and Miranda rivers (Table 1). Water temperature, dissolved O_2, pH, free CO_2, and dissolved organic carbon showed the greatest differences. Increases in conductance and total nitrogen were also recorded. Other variables such as chlorophyll *a*, phosphorus, and alkalinity did not show consistent changes during O_2 depletion. The concentration of major ions increased during oxygen depletion events in the Paraguay River, but not in the Miranda River. In general, concentrations of inorganic and organic carbon were higher during oxygen depletion events.

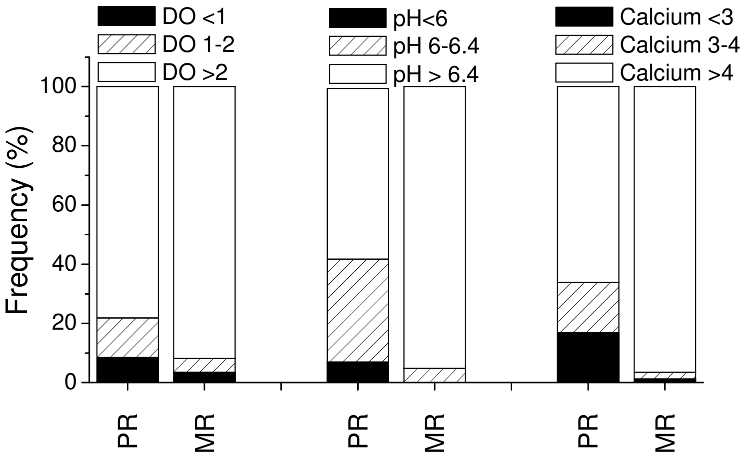

Figure 3. Frequency distributions of dissolved O_2 (mg l^{-1}), pH and calcium concentration (mg l^{-1}) grouped by classes indicating potential stress to *L. fortunei* as suggested by Ricciardi (1998). Data was taken in the Paraguay (PR-CR, N= 55) and Miranda rivers (MR, N= 44), respectively, from February 2003 to November 2007.

Table 1. Mean value ± SD of some chemical and physical variables of water from Paraguay and Miranda rivers when dissolved O_2 is less than or equal to 2.0 mg l^{-1} (oxygen depletion events) and higher than 2.0 mg l^{-1} (normal). Asterisks indicate that means are significantly different (5% significance, Kruskall Wallis test). Data were taken from 2000 to 2007 (Calheiros and Oliveira, unpublished data).

Variables	**Unit**	**Paraguay River –Corumbá**		**Miranda River**	
O_2 category		**≤ 2 mg l $^{-1}$**	**>2 mg l^{-1}**	**≤ 2 mg l $^{-1}$**	**>2 mg l^{-1}**
Number of samples		***18***	***79***	***11***	***77***
Water temperature	(° C)	28.5 ± 2.31	27.2± 3.6	30.2 ± 1.3	26.3 ± 3.4 *
Dissolved O_2	(mg l^{-1})	1.1 ± 0.7	5.4 ± 1.7 *	1.4 ± 0.6	5.7 ± 1.9 *
O_2 saturation	%	24.0 ± 24.5	69.2± 20.5*	18.8 ± 8.2	68.8 ± 23.3*
pH		6.4 ± 0.2	6.6 ± 0.4 *	6.7 ± 0.4	7.3 ± 0.4 *
Alkalinity	(µeq l^{-1})	431.5 ± 114.1	364.6 ± 88.8	1,389.8 ± 153.6	1,394.8 ± 258.2
Free CO_2	(mg l^{-1})	23.4 ± 10.6	13.7 ± 13.9 *	31.2 ± 25.6	13.9 ± 25.2 *
Conductance	(µS cm^{-1})	57.7 ± 10.5	46.5± 6.1 *	146.4 ± 15.8	139.6 ± 5.4
Chlorophyll *a*	(µg l^{-1})	1.2 ± 1.6	1.1± 1.6	0.9 ± 0.9	0.9 ± 1.2
Total nitrogen	(µg l^{-1})	732.5 ± 331.3	506.6 ± 221.5*	604.5 ± 145.4	581.6 ± 391.3
Total phosphorus	(µg l^{-1})	68.8 ± 40.7	42.0 ± 23.8	75.3 ± 23.2	76.6 ± 49.2
TOC	(mg l^{-1})	1.7± 1.2	1.1± 0.7	1.1 ± 0.4	1.8 ± 0.9
DOC	(mg l^{-1})	21.6 ± 9.6	14.0±11.8*	16.6 ± 13.9	14.0 ± 9.8
DIC	(mg l^{-1})	26.8 ± 3.2	23.5± 4.2	57.7 ± 20.3	62.0 ± 13.2
Ca^{2+}	(mg l^{-1})	5.5 ± 2.4	4.0± 1.9 *	15.8 ± 2.0	15.5 ± 4.6
Na^+	(mg l^{-1})	1.7± 0.3	1.9± 0.5	2.4 ± 0.3	2.4 ± 0.6
K^+	(mg l^{-1})	2.6 ± 1.0	2.0±0.7*	2.6 ± 1.0	2.2 ± 0.7
Mg^{2+}	(mg l^{-1})	2.2 ± 0.3	1.9 ± 0.7*	4.6 ± 0.6	5.8 ± 6.5
Mn^{2+}	(mg l^{-1})	0.1 ± 0.1	0.0± 0.0*	0.0 ± 0.0	0.0 ± 0.3
Fe^{2+}	(mg l^{-1})	1.1 ± 0.6	0.7± 0.6*	0.6 ± 0.3	0.4 ± 0.5
SO_4^{2-}	(mg l^{-1})	9.1± 7.2	7.6 ± 0.6	9.5 ± 7.9	10.0 ± 16.6

The effect of oxygen depletion events on densities of juveniles and adults

The probability of *L. fortunei* recruitment, based on density of juveniles, in water with nearly 0.0 mg l^{-1} of dissolved O_2 was less than 10% when the water temperature was about 32.0°C, a common situation from March to April (Figure 4). In waters with about 6.0 mg l^{-1} of O_2 the probability of recruitment was up to 80%, but close to 0% when temperature was lower than 20.0°C, as is common from June to September.

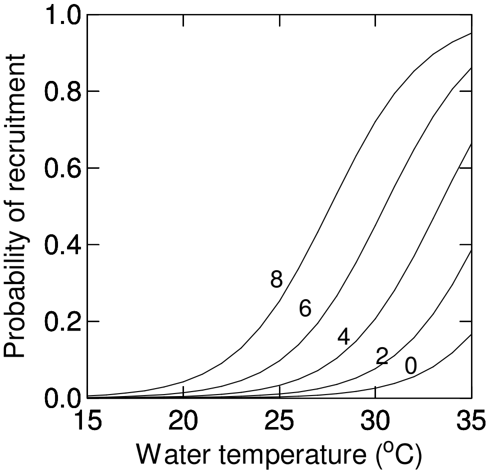

Figure 4. Trends of *L. fortunei* recruitment as a function of water temperature (°C) and dissolved O_2 (mg l^{-1}) based on a two-factor logistic regression model. Water temperature values were fixed and probability of recruitment was calculated for different O_2 concentrations (numbers along curves: 0.0, 2.0, 4.0, 6.0, and 8.0 mg l^{-1}). Data for water temperature, dissolved O_2 and juvenile density were collected from February 2004 to November 2007 in the Paraguay and Miranda rivers.

The annual range of juvenile density decreased from 2004 to 2007 in the Paraguay River and increased in the Miranda River, and the mean annual densities of adults dropped also from 2003 to 2007 in the Paraguay River and connected lakes (Figure 5 and Figure 6, respectively). Adults of *L. fortunei* covered about 60-70% of monitored rocks in the first two or three years and decreased to less than 10% in 2006 and 2007, including Castelo Lake, in which *L. fortunei* covered about 70% of rocks in 2005.

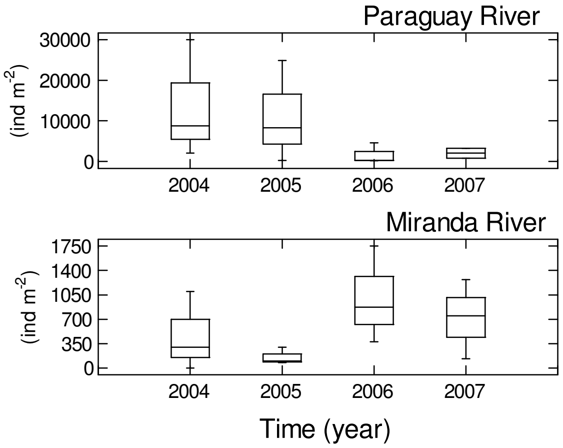

Figure 5. Densities of juvenile of *L. fortunei* from 2004 to 2007 in the Paraguay and Miranda rivers. Data were taken from January to March in the Paraguay River and from October to December in the Miranda River.

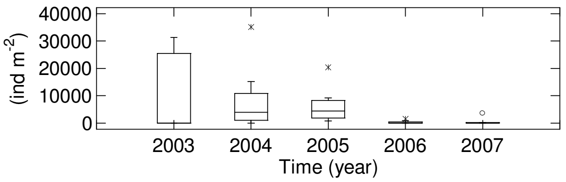

Figure 6. Densities of adult *L. fortunei*. Data are from samples collected during the low water phase, from 2003 to 2007, in the Paraguay River and connected lakes. In a box plot, the central horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall, with the box edges at the first and third quartiles.

Mortality of *L. fortunei* in oxygen depletion events

A. Field tests

Both *in situ* tests of *L. fortunei* tolerance to the oxygen depletion events showed that no mussels survived in Castelo Lake after the April 2006 and 2007 oxygen depletion events, which were similar in intensity to each other (Figure 7). From February to April 2006, O_2 dropped from ~6.0 to 0.0 mg l^{-1}, pH from ~7.0 to 5.2, $SI_{calcite}$ from -2.2 to -4.5, and free CO_2 rose from ~6.0 to 282.0 mg l^{-1}. Between February and May 2007, dissolved O_2 decreased from ~6.0 to 0.0 mg.l^{-1} and pH from ~7.2 to 5.0-5.5, free CO_2 reached 120.0 mg l^{-1} and $SI_{calcite}$ reached -4.0. The water temperature ranged from 27.0 to 34.0°C in both periods. In contrast, the mussels survived and grew in lake water between the oxygen depletion events. After 200 days immersed in normal water conditions, between both oxygen depletion events, the average shell growth rate was 0.05 mm day $^{-1}$, with shell length reaching about 18.0 mm in the first year.

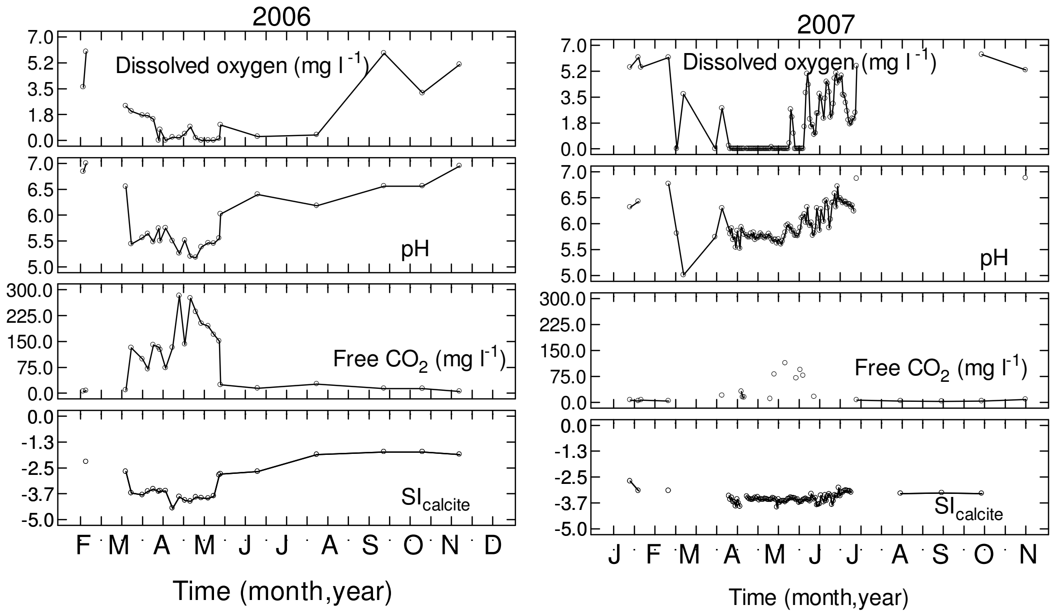

Figure 7. Variation of dissolved O_2 (mg l^{-1}), pH, free CO_2 (mg l^{-1}) and SI$_{calcite}$ during the 2006 (left graphic) and 2007 (right graph) oxygen depletion events in Castelo Lake.

B. Laboratory tests

Temperature effects

In laboratory assays lasting for up to 70 hours at 26.0°C no mortality was recorded in water from the oxygen depletion event, nor in the control water. At 33.0 ± 1°C around 30-40% of mussels died in oxygen depletion event water and less than 10% died in control water (Figure 8). At 35.0 ± 1°C, all mussels died in oxygen depletion event water by 50 hours of exposure, and about 50% died in control water. All treatments had O_2 higher than 1.0 mg l^{-1}, pH higher than 7.0 and calcium greater than 7.0 mg l^{-1}. Initial conditions are shown in the figure 7 (2007 data, right graph). Dissolved O_2 and pH were higher during laboratory tests because water from oxygen depletion events underwent some aeration when it was transported to the laboratory.

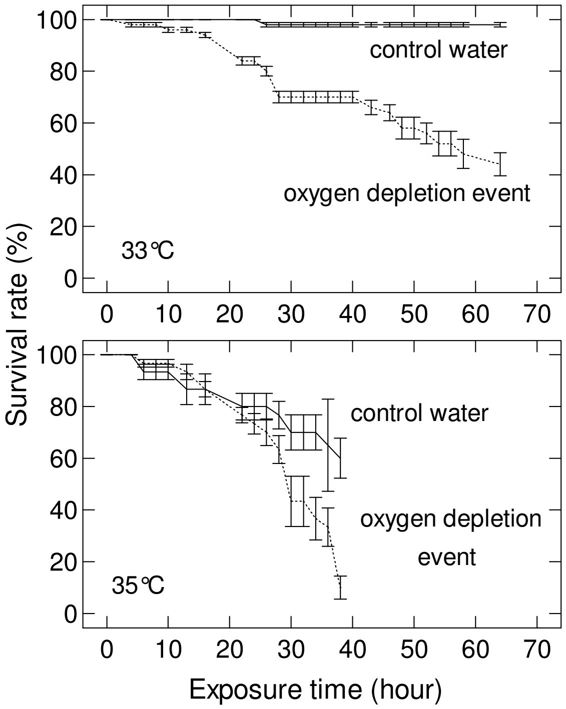

Figure 8. Survival of *L. fortunei* adults incubated in Castelo Lake water, taken during the 2007 oxygen depletion event (dashed line), compared to control water from a laboratory tank (solid line), at two water temperatures.. Lines represent the mean and bar is the standard error around the means.

Organic compound effects

Survival tended to be lower ($F_{(1,141)}$= 179.8, p= 0.00) in water from the oxygen depletion event compared to the DOC-free water (Figure 9). Water taken from the Paraguay River had the following conditions upon sampling: O_2 0.2 mg l^{-1}, pH 6.3, and calcium 8.1 mg l^{-1}. At the laboratory water from the oxygen depletion event had changed due to the aeration as explained before: O_2 had risen to 6.8 mg $l^{-1}$and pH to 6.5. DOC-free river water had dissolved O_2 at 4.7 mg l^{-1} pH of 6.9 and calcium at 18.4 mg l^{-1}. During the experiment dissolved O_2 oscillated between 3.0 and 5.0 mg l^{-1}. Values of pH were above 6.5 for the both treatments, increasing slightly by the end of the experiment.

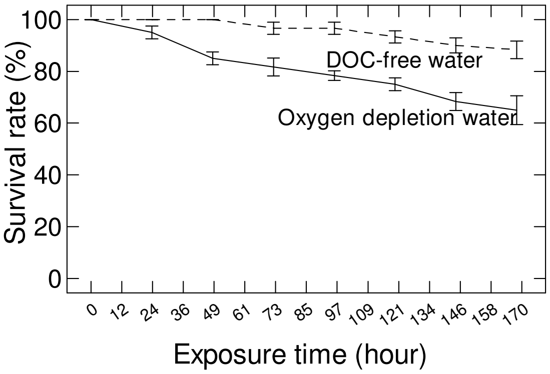

Figure 9. Survival of *L. fortunei* adults during 170 hours of exposure to water from an oxygen depletion event (solid line) and the same water with no organic compounds ("DOC-free water", dashed line), at 32°C. Lines represent means of three replicates for each treatment.

Decalcification assays

Results from the decalcification assays are summarized in Table 2. In the water from Castelo Lake that was used for the assays, the mean initial O_2 was 3.8 mg l^{-1} in normal water and 1.5 mg l^{-1} in water from the oxygen depletion event. The concentration of O_2 fell during the assays, and in water from the oxygen depletion event this was accompanied by measurable decreases in pH. The rate of consumption of O_2 was about 2-fold higher in water from the oxygen depletion event, whereas CO_2 production (calculated from pH) was more than 20-fold higher. The molar ratio of O_2 consumed to CO_2 produced indicated that aerobic respiration predominated in normal water, whereas substantial excess CO_2 production took place in water from the oxygen depletion event, presumably reflecting anaerobic metabolism. Increases in total alkalinity and dissolved Ca^{2+} concentrations indicated that net decalcification took place in most of the assays (a few showed no significant changes), with the largest increases observed in the O_2 depleted water. Roughly similar rates of decalcification were estimated from increases in alkalinity and Ca^{2+}, with both measures indicating that decalcification rates were ~7-fold higher in the O_2-depleted water. Non-significant slopes in most of the assays without mussels indicated that no significant changes occurred.

Table 2. Mean values (standard errors in parentheses) for decalcification assays performed in a closed-system with *L. fortunei*, using normal and oxygen-depleted event water from Castelo Lake.

	Normal water (N = 3 assays)	**O_2-depleted water (N = 6 assays)**
Initial O_2 (mg l^{-1})	3.81(0.09)	1.49 (0.33)
Initial pH	7.00 (0.01)	6.32 (0.08)
Final pH	6.99 (0.03)	6.24 (0.08)
O_2 consumption (mg l^{-1} h^{-1})	0.15 (0.13)	0.33(0.16)
CO_2 production (mg l^{-1} h^{-1})	0.09 (0.09)	1.98 (0.89)
Molar ratio $\Delta O_2/\Delta CO_2$	0.71 (0.71)	0.12 (0.09)
Alkalinity increase (meq l^{-1} h^{-1})	0.0015 (0.0015)	0.0107 (0.0049)
Equivalent Ca dissolution (µmol g^{-1} wet weight h^{-1})	0.033 (0.030)	0.200 (0.090)
Dissolved Ca^{2+} increase (µmol l^{-1} h^{-1})	1.17 (1.17)	8.38 (1.91)
Equivalent Ca dissolution (µmol g^{-1} wet weight h^{-1})	0.047 (0.047)	0.335 (0.076)

Discussion

The density of *L. fortunei* in the Pantanal is low compared to other places in South America where they have been measured. Adult density on natural substrata in the Paraguay River was about 35,000 individuals m^{-2}, restricted to areas with rocks, while in the Guaiba Lake (Guaiba River system in Southern Brazil), *L. fortunei* reaches 100,000 individuals m^{-2} on roots (Mansur et al. 2003) and in the Paraná River (Argentina) its reported density was about 150,000 individuals m^{-2} on artificial surfaces (Pastorino et al. 1993; Darrigran et al. 2003). It is difficult to compare densities among places due to the different methodologies, but observations from researchers confirm that in the Guaiba Lake and the Paraná River *L. fortunei* exhibits dense aggregations with most of the substratum covered by mussels in more than one layer. In the Paraguay River, generally, the aggregations are in a thin layer and the substratum is not completely covered by mussels; we observed a maximum 70% coverage of the surfaces, restricted to certain places with adequately hard surfaces.

A sequence of stressful factors, such as low water levels that killed mussels by exposing them to the air and low pH during the falling water, probably reduced the densities of juveniles and adults, but this study has shown that depletion of O_2 alone during rising water is a very important variable controlling mussel density, and sometimes can even prevent its establishment.

Oxygen depletion can affect *L. fortunei* in two ways, precluding spawning or killing larvae, juveniles, and adults (Morton 1975). Interannual fluctuation in abundance was essentially related to the intensity and duration of oxygen depletion events, and mainly in the Miranda River it is affected by the time of year, because oxygen depletion events might occurs between December and February and halt the spawning before seasonal decreases in temperature take place. In the Paraguay and Miranda rivers it is probable that *L. fortunei* recruitment decreases from February to April, at the end of the reproductive period, due to lower dissolved O_2 and then it is again negatively affected from May to September by the lower water temperatures.

The most important effects during oxygen depletion events in the Pantanal wetland must be attributed to simultaneous stress factors including low O_2, low pH, and high free CO_2 under high temperature. Results from this study showed that *L. fortunei* was not tolerant to simultaneous conditions approaching 0.0 mg l^{-1} of O_2, pH 5.0, $SI_{calcite}$ of -4.0 and free CO_2 above 100 mg l^{-1}, in water temperatures about 30.0°C. These conditions were recorded during the oxygen depletion events in the Castelo Lake. The population that covered about 70% of rocks in 2005 was completely extirpated during the 2006 oxygen depletion event as a result of this stressful combination of factors.

L. fortunei can establish itself in the Castelo Lake between oxygen depletion events, and in those intervals shell growth rate was similar to those observed in other environments (Boltovskoy and Cataldo 1999; Oliveira et al. in review). Thus, oxygen depletion events may be a primary control of *L. fortunei* establishment and density, especially in lentic systems. *L. fortunei* has been observed to survive to some depletion of O_2 (close to 1.0 mg l^{-1}) at the bottom of the Plover Cove reservoir (Hong Kong) where no mortalities were recorded but the densities were lower than on shallower sediments with more oxygenated overlying water (Morton 1977). Intolerance to hypoxia, particularly at high temperatures, has been reported in North America for two other introduced mussels: *Dreissena polymorpha* (Yu and Culver 1999; Mihuc et al. 1999) and *Corbicula fluminea* (Johnson and McMahon 1998; Matthews and McMahon 1999; Saloom and Duncan 2005).

Corbicula fluminea is native to the same region as *L. fortunei* (Morton 1996) and occurs in the Pantanal wetland since 1990 (Callil and Mansur 2002). According these authors, as well as *L. fortunei,* C. *fluminea* presents lower densities in the Pantanal wetland compared to other South American habitats. According Callil and Mansur (2002) lower density of these species can be associated to the time of introduction and environmental conditions. *C. fluminea* and *L. fortunei* are typical freshwater river species and might withstand the anoxic conditions in rivers better than in lakes, and probably they have increased their ability to regulate the oxygen consumption under hypoxic or anoxic events as suggested by Chen et al. (2001), and have been persistent in the Pantanal floodplain even in low densities.

Under laboratory conditions, mortality of *L. fortunei* in oxygen depleted water increased with temperature, suggesting that high temperature in the Pantanal wetland exacerbates the impact of oxygen depletion events, perhaps by reducing the ability of mussels to regulate the consumption of O_2 under hypoxic conditions, as observed by Chen et al. (2001) in zebra mussels.

We did not find information about the combination of hypoxia/anoxia and changes in pH or free CO_2 as observed in the Pantanal waters, where oxygen depletion is normally accompanied by decreased pH, reflecting increased free CO_2 produced by the respiratory metabolism of microbes and plants. In waters of the Pantanal, Hamilton et al. (1997) have speculated that in addition to aerobic bacteria, root respiration by vascular plants and O_2 utilization by methane-oxidizing bacteria may also be significant to overall oxygen consumption and CO_2 production.

Mortality of *L. fortunei* during oxygen depletion events can be associated with other chemical factors such as acidification and shell decalcification, and organic compounds. Hamilton et al. (1995) concluded that net ecosystem metabolism in Pantanal waters is strongly heterotrophic, based on dissolved gas concentrations, which typically showed depletion of dissolved oxygen and considerable supersaturation of CO_2 in the floodplain waters. Assays using input of CO_2 from mussels' respiration corroborated the idea that excess of CO_2 must contribute to the water acidification and calcium dissolution observed during oxygen depletion events in Pantanal waters.

Evidence of calcium loss was given by field records about the fast shell decalcification of *L. fortunei* during oxygen depletion events, as also observed by Morton (1975) in Plover Cove reservoir, which related the fast decalcification to the low calcium concentration. Additionally, mussels from the Paraguay River, especially from the Corumbá site, where calcium concentration is slightly lower than in other sites, have thin and fragile shells, while mussels from the Miranda River have thick and heavy shells, although decalcification was also observed in the Miranda River during oxygen depletion events. It is likely that *L. fortunei* lost calcium to maintain the ionic equilibrium in waters with low calcium; because acclimation might promote some adjustments (Deaton et al. 1989). Under stressful conditions, as during oxygen depletion events, the ability of *L. fortunei* to maintain its ionic equilibrium in low calcium concentrations might be altered, and this may be another possible cause of death.

L. fortunei also lives in low calcium concentration, between 1.0 to 6.0 mg l^{-1} (average 3.6 mg l^{-1}) according to our studies. These values are low compared to the native environments (Oliveira et al. in review) confirming the wide capacity of adaptation of this species. Estimates of calcite saturation using data from Zhang et al. (2007), from Pearl River ($SI_{calcite}$= 1.39 to -0.35) and its tributary, East River ($SI_{calcite}$= -1.43 to -1.63), showed that the natural environment of *L. fortunei* is also undersaturated, although resembling more the Miranda River ($SI_{calcite}$>-2) than the Paraguay River ($SI_{calcite}$< -2.0). At the Paraguay River, especially during oxygen depletion events, *L. fortunei* might be subjected to calcium limiting conditions and some mortality can take place.

In the Pantanal, *L. fortunei* usually lives in water where the minimum pH is around 6, although laboratory tests conducted by Montalto and Marchese (2003) demonstrated that *L. fortunei* might live in pH as low as 5 for about 10 days. Tolerance to low pH and low ion concentration outside its natural environment seems related to the ability to maintain ion balance, since *L. fortunei* has high capacity of osmoregulation (Deaton et al. 1989). These authors verified that survival of *L. fortunei* in deionized water or water up to 200 mOsm was higher than 80% for more than 3 weeks, and for at least 63 days. Mussels had byssal attachments and were filtering by the end of first week. In diluted waters *L. fortunei* increases the level in the calcium hemolymph (Deaton et al. 1989); similar results were found by Mackie and Flippance (1983) who found some mussels living in pH <5, and some of them were able to concentrate calcium from waters with low or no buffering capacity, and likely might use the calcium shell reserve when environmental conditions are unfavorable (Araujo et al. 1994).

Other components in oxygen depletion events water that could affect *L. fortunei* are aquatic humic substances. They generally comprise one-third to one-half of the dissolved organic carbon (DOC) in water, and rarely exceed 5.0 mg l^{-1} (Thurman 1981; Wetzel 2001). Dissolved organic carbon in the Paraguay River was higher compared to the other large rivers with floodplains such as Orinoco (mean of 4.4 mg l^{-1}, Lewis et al. 1990) and Amazon rivers where DOC ranges from 1.4 to 13.1 mgl^{-1} (Walker 1995), including black water from Rio Negro where waters are completely acid (pH= 5.0-6.0, DOC= 10.0-15.0 mg l^{-1}).

In despite of DOC importance as a source of mollusk food; Roditi et al. (2000) suggested that DOC contributes up to 50% of the carbon demand for *Dreissena polymorpha*, we suspect that increase of humic substances (HS) on DOC composition during rising water phase (Hamilton et al. 1997), have some importance on mussels mortality. *L. fortunei* probably concentrates a large quantity of this substance due the high filtration rate (Sylvester et al. 2005). Steinberg et al. (2003 and 2008) have postulated that HS are taken up by freshwater organisms, and it can work as xenobiotic chemicals (Pflugmacher et al. 2001; Steinberg et al. 2003 and 2008; Euent et al. 2008). Poor mussel survival in water DOC-free experiments suggests that presence of organic compounds might also contribute to the drastic mortality observed in Castelo Lake.

Other factors that could contribute to the mortality of *L. fortunei* during oxygen depletion events were analyzed by Hamilton et al. (1997), and Calheiros and Hamilton (1998). They measured 48 trace elements from 1993 to 1995 and found that during oxygen depletion events these elements did not greatly exceed their previously observed ranges and probably did not reach toxic levels in the Pantanal waters, so they are not responsible for mussel mortality. Other reduced substances produced during decomposition such as ammonia, H_2S, Al, NO_2^-, NO_3^- showed low concentrations and have no significant increments during oxygen depletion events in Castelo Lake (Calheiros and Hamilton 1998). No algal blooms were evident during oxygen depletion events from 2003 to 2007, nor from results by Calheiros and Hamilton (1998). Results from this study for some elements such as Fe, Mn, Mg, Na, and K did not show differences during oxygen depletion events, either in the Paraguay and Miranda rivers or in Castelo Lake.

Beyond the influence of the floodplain

Of the five lakes linked to the Paraguay River we studied, only in Castelo Lake we observed mussel eradication. Dissolved gases (low O_2 and high free CO_2), presence of non-specified organic compounds combined with acid waters were suggested to control *L. fortunei* establishment at this place. In the other lakes we observed decreasing density in the last years, as well in the Paraguay River, related to the more intense oxygen depletion events in the last years. At Uberaba Lake, the largest lake linked to the Paraguay River, we found an installed population with individuals reaching 40 mm length, a size not so common in other parts of the Paraguay River and connected lakes, where the shell is usually about 20 mm (Santos et al. 2004, Santos et al. 2005). Information from local people indicates that oxygen depletion events are not usual there, and mussels can live more than one year and grow longer shells. Places like Uberaba Lake work as a reservoir of *L. fortunei* larvae and maintain viable populations able to spread laterally and downriver, especially during inundation, when the river communicates with its large floodplain. Additionally, in the Paraguay or Miranda rivers oxygen depletion events did not eliminate *L. fortunei*, the effects were restricted to controlling its density. Castelo Lake might be colonized again by *L. fortunei* in the following flooding, if oxygen depletion events are not strong enough to kill them. Although oxygen depletion events can eliminate *L. fortunei* in the connected lakes if it is subjected to oxygen depletion conditions as in Castelo Lake, it did not preclude its spread to the north of the Paraguay River or laterally to tributaries such as the

Miranda River. Beyond the influence of the floodplain there is no record of oxygen depletion events and *L. fortunei* probably will have great success colonizing Paraguay River tributaries, given the similarity of physical and chemical conditions in the UPB, and the species tolerance to an ample range of variables. Therefore, without this event *L. fortunei* probably would have colonized much more habitats at a greater speed and higher density than it is observed now.

Capítulo 3

Modeling the potential distribution of the golden mussel *Limnoperna fortunei* in the upper Paraguay basin and other Brazilian rivers using limnological variables.

Abstract

The bivalve *Limnoperna fortunei* (Dunker, 1857), also called golden mussel, was introduced into the La Plata River estuary (South America) and quickly expanded upstream to the north, reaching Pantanal wetland, Brazil. In the upper Paraguay basin *L. fortunei* occurs along the course of the Paraguay River, most of its connected lakes and in three tributaries, although restricted to the lower portion of these tributaries. Based on current knowledge about the limiting factors for golden mussel establishment and on chemical and physical water characteristics of the receptor environment we forecasted its potential geographic distribution in the upper Paraguay basin and other Brazilian river systems. We used the spatial distribution of calcium concentration and $SI_{calcite}$, the genetic algorithm for rule-set production (GARP) best subset approach, and the maximum entropy method (Maxent) to model the geographic distribution of *L. fortunei*, using presence-only data. The variables used to build the environmental layers were water temperature, dissolved oxygen, pH, calcium, $SI_{calcite}$ and total suspended solids (TSS). Important tributaries of the Paraguay River such as Cuiabá and Miranda/Aquidauana rivers exhibit high risk of invasion and should be monitored carefully. Our results also suggest that large portions of the Brazilian rivers are able to be invaded by *L. fortunei*.

Introduction

Almost 95 % of the international business in Brazil is transported via oceanic vessels. About 40 million tons of ballast water are discharged into Brazilian coast from about 40,000 international ships that visit the seven Brazilian harbors annually (Silva and Souza 2004). Although ocean or freshwater systems have received several exotic species, only a small portion of these organisms have succeeded in establishing themselves and, among these, only part are considered a plague, responsible for economic and environmental losses, and the costs are difficult to measure (Moyle and Light 1996).

Two mollusk species, supposedly introduced by ballast water, have established themselves in the American freshwater systems causing numerous environmental problems, as well as additional economic costs: the zebra mussel *Dreissena polymorpha* (Pallas 1771) which 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 (O'Neill 1997; Strayer et al. 1999; Schloesser and Nalepa 1994), and the golden mussel *Limnoperna fortunei* (Dunker 1857), established in South America in the beginning of 1990 with similar impacts (Darrigran and Escurra de Drago 2000; Boltovskoy et al. 2006; Oliveira et al. 2006; Karatayev et al. 2007).

In order to estimate the potential geographic distribution of invasive species, many studies have considered the means of transport to a new location, and their establishment and integration into the new environment (Moyle and Light 1996; Shea and Cheson 2002; Karatayev et al. 2007). The fast expansion of zebra mussels in North America has resulted from a combination of processes such as propagule spread via navigation (Brossemboek et al. 2007; Colautti et al. 2006) as well as physical and chemical characteristics of the environment providing chances to grow and reproduce (Ramcharan et al. 1992). Other biological characteristics such as byssal threads and a larval phase (veliger), that favor respectively attachment and dispersal, have contributed to the expansion of *D. polymorpha* (Carlton 1993; Johnson and Carlton 1996; Johnson and Padilla 1996; Bobeldyk et al. 2005).

A similar association of factors has been observed concerning the expansion of *L. fortunei* in South America, in the La Plata Basin. Its success in the upstream colonization is mainly related to the regular boat traffic along the Paraguay-Paraná waterway, which includes barge trains that readily transport organisms. Dispersal from the main river channel to the tributaries is slower due the lower boat traffic (Boltovskoy et al. 2006; Oliveira et al. in review). According to Boltovskoy et al. (2006) *L. fortunei* responds for two basic mode of geographic expansion: a gradual dispersion from a localized epicenter and a wave-like dispersion punctuated by long distance transport events or '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). They defined ecological niche as the set of environmental factors that determine where a species can and cannot maintain populations. Modeling based on ecological niche may allow identification of broader ranges 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 has been used to assess potential geographic distributions of non- native species when a good database is available qualitatively and quantitatively, for a realistic prediction (Peterson and Vieglais 2001; Peterson 2003). Two examples are the prediction of expansion of *D. polymorpha* in North America (Bossembroek 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. Global scale analyses and the routes of introduction have shown that *L. fortunei* is likely to occur in most waters of South America, including the Amazon, Orinoco and Magdalena river systems, where this species has not been recorded up to now. It has been proposed that *L. fortunei* may colonize warm waters in North America, both by comparing the species requirements and chemical characteristics of water (Ricciardi 1998; Boltovskoy et al. 2006; Oliveira et al. (in preparation), and by modeling using climatological and topographic datasets (Kluza and McNyset 2005). These models, however, do not provide detailed information in small geographic scales for aquatic species.

For smaller scale distributions, such as hydrographic basin, 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), because occurrence and average density were highly correlated with these water chemistry variables. Low concentrations of calcium and low pH are suspected to influence density or restrict the establishment of bivalves since in most mollusks shell formation is related to the available calcium in the environment (Mackie and Flippance 1983; Ramcharan et al. 1992; Checa et al. 2007).

In this study we forecasted the expansion of *L. fortunei* in a small spatial scale, applying physical and chemical aquatic variables that are not commonly used in wide spatial scale models. We used spatial distribution of calcium concentration and $SI_{calcite}$, and ecological niche modeling to predict the geographic expansion of *L. fortunei* in the upper Paraguay basin, and we then used this experience to project the potential distribution to other Brazilian rivers systems. Here we did not evaluate introduction routes or other anthropic factors; rather, we focused on the river's abiotic potential to be invaded by *L. fortunei*. The invasion of *L. fortunei* in Brazil after 10 years might be regarded as small compared to the extension of Brazilian river systems, so there is still time and opportunity to establish preventive programs in the regions with higher risk of *L. fortunei* colonization should it be introduced there. 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.

Methodology

Study area

Brazil is the fifth largest country in the world, representing 47.7% of all the South American territory, with an area slightly larger than 8.5 million km^2. It is privileged in terms of water resource availability. The mean annual river discharge in the Brazilian territory lies in the order of 267,000 m^3/s, that is, about 18% of the world's availability, if watersheds originated in neighbor countries are considered (Amazon, 86,321 m^3/s, Uruguay, 878 m^3/s and Paraguay, 595 m^3/s) (MMA 2007).

In terms of climate, Brazil is subjected predominantly to hot temperatures (92% of the country is located in the intertropical zone, with mean upper temperatures higher than 20ºC) with abundant rainfall (above 2,500 mm/yr), prevalent in the Amazon region. The majority of rivers are perennial and formed by rainfall. Regarding topography, Brazil has mostly modest altitudes, with a predominance of variations of plateaus, plains and valleys, compatible with a territory constituted basically from rather ancient geological structures (Paleozoic to Mesozoic). It has, however, some basins of recent sedimentation, that correspond to the Pantanal Sul-Matogrossense, part of the Amazon basin, and parts of the northeastern and southern littoral. For planning and managing purposes, Brazil adopted, in its Water Resources National Plan (Plano Nacional de Recursos Hídricos), a division of the country in 12 Hydrographic Regions: Amazon, East Atlantic, West Northeastern Atlantic, Eastern Atlantic, Southeastern Atlantic, Southern Atlantic, Parnaíba, São Francisco, Tocantins-Araguaia, Uruguay, Paraguay, and Paraná, organized according to the location of the main Brazilian hydrographic basins (MMA 2007).

The upper Paraguay basin (UPB), Paraguay region, covers about 496,000 km^2, within which the Pantanal wetland covers about 160,000 km^2, 140,000 km^2 belonging to Brazil, 15,000 km^2 to Bolivia and 5,000 km^2 to Paraguay (Junk et al. 2006). The Pantanal is an extensive floodplain surrounded by plateau areas, a fragile and complex system dependent on the flow regime (flood pulse) and water quality of the Paraguay River tributaries.

The Paraguay River runs from north to south collecting the waters of large tributaries such as Jauru, Cabaçal, and Sepotuba rivers in the right margin, and Cuiabá (with its tributaries São Lourenço and Piquiri rivers), Taquari, Negro and Miranda (with its tributary Aquidauana) in the left margin. Also on the right margin, there is an extensive series of river-connected lakes surrounded by higher lands (Hamilton et al. 1997; PCBAP 1997). Our study area included the Paraguay River and its major tributaries (Figure 1), and connected lakes.

The Paraguay River fluctuation is from 2 to 5 m during the year. Flooding (rising stage) in the Paraguay River occurs from January to May, followed by high water before August. Generally, the floodplain water levels are shallow (1 to 2 m) but with substantial flow. The lakes are shallower than the rivers, which are deep about 3-6 m, but usually retain water during the dry season (Hamilton et al. 1995). Most connected lakes along the Paraguay River exchange water with it and can contribute to changes in river chemistry particularly during falling and rising water periods. The effects of the extensive floodplain on the biogeochemistry of the Paraguay River were described by Hamilton et al. (1997) and Calheiros and Hamilton (1998) and Calheiros (2003). Those effects drive the structure and density of aquatic organisms such as phytoplankton (Oliveira et al. 2006), fish (Calheiros et al. 2000), zooplankton (Brandorff et al. submitted) and benthos (unpublished data).

Waters of the Pantanal are poor 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 by agricultural activities in the plateau regions increases sediment input along the rivers course, and massive agricultural activities introduce nutrients and pesticides to the water, which can reach the floodplain (Miranda et al. 2008). In the floodplain extensive cattle ranching is the main economic activity, followed by amateur 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.

Data collection and analysis

The potential distribution of *L. fortunei* in the UPB and the other Brazilian river system was predicted in three different ways: through an exploratory analysis based on the spatial distribution of calcium concentration and $SI_{calcite}$, and by applying two niche models that have been used to predict alien species invasions: GARP (Genetic Algorithm for Rule-set Prediction) and Maxent (Maximum Entropy Method).

GARP is an algorithm that creates ecological niche models for species, based on presence data. The models describe environmental conditions under 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 Peters 1999). We used GARP best subset 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).

Maxent is also based on presence records (Phillips et al. 2006). According to Phillips et al. (2006) the idea of Maxent algorithm is to estimate the geographic distribution of species from locality point 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. Maxent was ranked among the most effective methods for species distribution modeling from presence-only data in a recent comprehensive model comparison study (Elith et al. 2006). The software is available at http://www.cs.princeton.edu/~chapire/maxent, version 3.0.4 beta.

All predictions were made based on the following limnological variables: water temperature, dissolved oxygen, conductance, pH, calcium concentration, and calcite saturation index ($SI_{calcite}$) and TSS. $SI_{calcite}$ was used to understand the thermodynamic propensity for calcification of the waters, and it is a combination of water temperature, pH, conductance, total alkalinity (Gran 1952) and dissolved calcium, calculated based on APHA (1998). 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. Except for $SI_{calcite}$, all other variables have been used to predict the distribution and density of *D. polymorpha* in North America (Ramcharan et al. 1992; Neary and Leach 1992; Mellina and Rasmussen 1994). Turbidity was used to predict zebra mussel spread in large rivers like the Mississippi, although the effects were observed when combined with high temperature

(Alexander et al. 1997; Allen et al. 1999), and here we use total suspended solids (TSS) in the UPB as a variable related to the turbidity. Chlorophyll *a* was used to compare food availability between different aquatic systems.

One critical point is to decide which explanatory variables, or combination of variables, should enter the model. There are different methods to help define these variables, including some statistical analysis (Guisan and Zimmerman 2000). In our case we ran models with different combinations of variables. We did not find expressive difference between the results and decided to compare a combination of all available variables against only one variable ($SI_{calcite}$) in a separate model.

No at 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), Oliveira et al. (in review), and Oliveira et al. (in preparation). 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. We applied the same criteria for both the UPB and the other Brazilian river systems. The relationship between calcium concentration and $SI_{calcite}$ is expected to be specific to a particular water, reflecting its hydrogeochemical composition and net ecosystem metabolism (a control on pCO_2). So, we used a linear regression model to estimate the relationship between calcium concentrations and $SI_{calcite}$ for the each Brazilian system, and to compare the $SI_{calcite}$ in different waters with 1.0 mg l^{-1} of calcium. Only values below 20.0 mg l^{-1} of calcium were used.

Data used to model the potential distribution in the UPB were taken from 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, which provided data from nine sampling sites in the Paraguay River, 114 in the most important tributaries of the Paraguay River basins, and seven sites in the connected lakes (Uberaba, Gaíva, Zé Dias, Mandioré, Castelo, Canal do Tamengo, and Baía Negra). We only used limnological samples taken 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 Cuiaba rivers during 2007 oxygen depletion events.

The Brazilian river systems data base was taken from the 'Brasil das Águas' project (www.brasildasaguas.com.br) and data from Embrapa Pantanal for UPB. We used 786 sampling sites located along six systems: Amazon, Paraná/Paraguay, São Francisco, Tocantins-Araguaia, North Atlantic (East Atlantic, West Northeastern Atlantic, Eastern Atlantic) and South Atlantic (Southeastern Atlantic, Southern Atlantic). Except for Paraguay system, each other site was represented by a one-time sample obtained between 2003 and 2004. We only considered environments with salinity below 2.0 ppt, according to the tolerance of *L. fortunei* (Darrigran 2002).

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 and Brazilian river systems. Environmental layers were built using the software Arc View 3.2 (ESRI 1999), following Latini (2006). A drainage map of the UPB, scale 1:100.000, was taken from the National Water Agency (Agência Nacional de Águas) and a map of Brazilian drainage was taken from Arc View 3.2 Software.

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), totaling 943 geographic points. In the case of Brazilian rivers we did not interpolate data due to the large spatial scale. From a total of 130 sites in the UPB, 24 sites had occurrence records, located in the Pantanal wetland (Figure 1). We used the same 24 records of occurrence for the Brazilian river systems' models, plus six sites in the Paraná River. We used 50% of this data for training and 50% for test in both GARP and Maxent models. Grid cells of 0.07 degrees were used in the case of UPB and 0.40 degrees in the Brazilian river systems analysis.

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; Philips et al. 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.

Results

Current *L. fortunei* distribution in the upper Paraguay basin

The current occurrence area of *L. fortunei* in the UPB is shown in Figure 1, which includes the results from Oliveira et al. (2004) and new information added after 2004. *L. fortunei* is restricted to the Paraguay River main channel, and adjacent inundation area, including connected lakes, and the lower and middle portion of Miranda and Apa tributaries (Oliveira et al. 2004). New records of *L. fortunei* after 2004 were observed in Uberaba Lake, a huge lake linked to the Paraguay River in the northern part of UPB, about 100 km upstream in the Miranda River (SEMAC/MS, personal communication), and in the estuary of the Cuiabá River (Figure 1). In the Cuiabá River the population density is very low, since only six mussels were counted.

Limnological characterization of upper Paraguay basin

The mean pH in the UPB is predominantly between 6.0 and 7.0 (Figure 2a). Some tributaries such as Jauru, Cuiabá, Miranda/Aquidauana and Apa have mean pH above 7 and in others, such as Piquiri, Taquari, and Negro pH might be as low as 5.0. Although the pH is above 6.0 in the upper section of the tributaries it can be below 6.0 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, the UPB waters are ion dilute, as in the Paraguay River, where conductance was around 50-60 $\mu S\ cm^{-1}$. More dilute waters were found in the São Lourenço basin (3.0 to 19.0 $\mu S\ cm^{-1}$), Sepotuba (10.0 to 20.0 $\mu S\ cm^{-1}$) and in the Taquari and Negro rivers (25.0 $\mu S\ cm^{-1}$). These rivers exhibited minimum calcium concentration around 0.0 mg l^{-1} (Figure 2b), and average of 2.0 mg l^{-1}. These rivers 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 -3.0 (Figure 2c). In general the calcium concentration in the Paraguay River is above 3.0 mg l^{-1}(Figure 2b), but may attain minimum values of 1.0 mg l^{-1}.

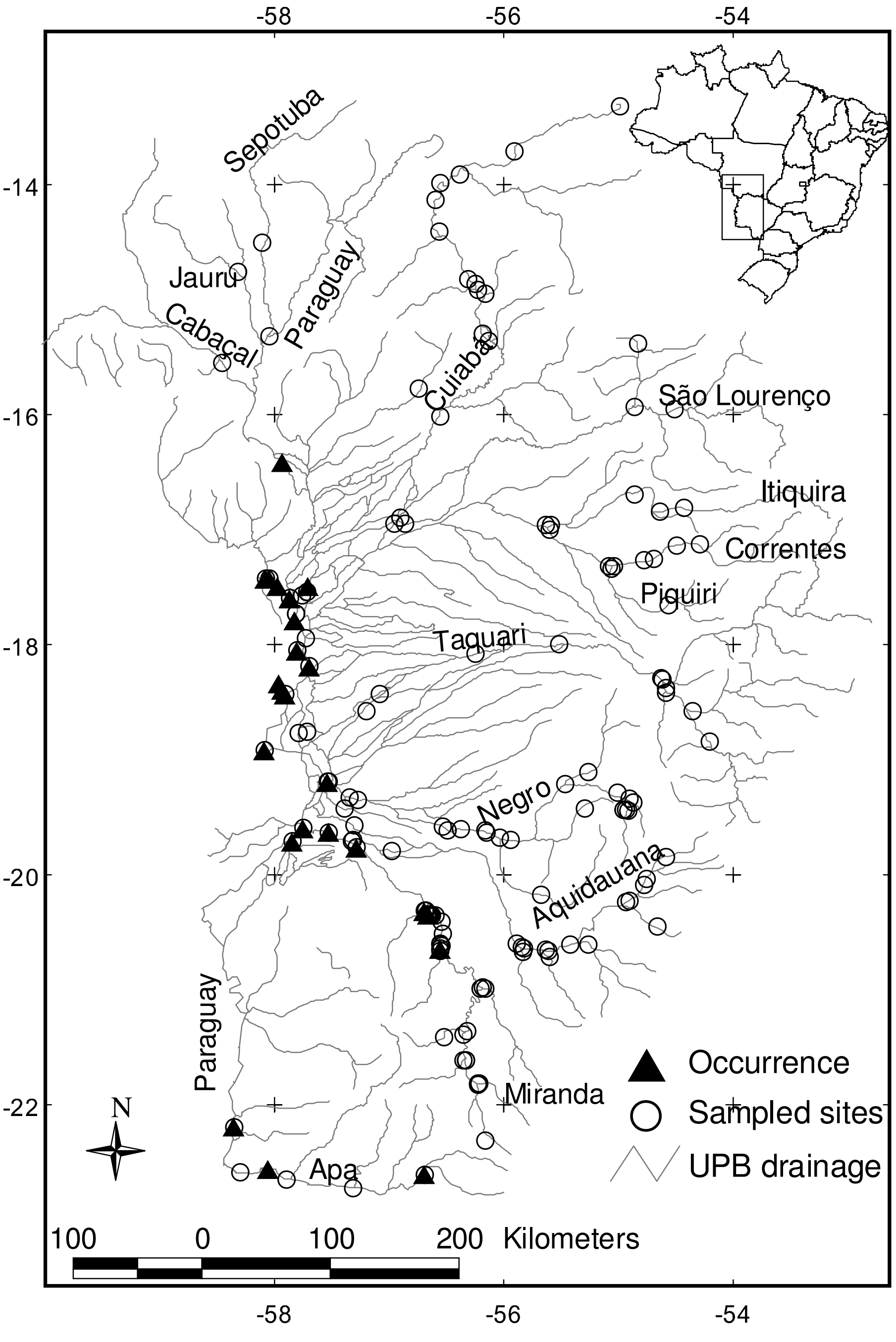

Figure 1. Current distribution of *L. fortunei* in the UPB until 2008 (full triangle). Open circles represent locations sampled for limnological variables. UPB drainage map source: ANA (National Agency of Water), scale: 1:100.000. Map built using Arc View 3.2 software.

In the upper Cuiabá River, the major tributary of Paraguay River, conductance was between 60.0 and 100.0 µS cm^{-1}, and in the lower section of the Miranda River was between 84.0 and 178.0 µS cm^{-1}. Other river systems such as Miranda River and Apa River presents some tributaries with conductance as high as 400-600.0 µS cm^{-1}. In these rivers, minimum calcium values were above 5.0 mg l^{-1} and reached around 60.0 mg l^{-1} (Figure 2b). These waters can be diluted with floodplain waters in their estuaries. In the floodplain area, the Cuiabá River receives water from the São Lourenço and Piquiri rivers, promoting dilution, and mean conductance was 38.0 µS cm^{-1}. Calcium concentration in the lower Cuiabá River was between 3.0 and 4.0 mg l^{-1}, but the minimum can be as low as 1.2 mg l^{-1}. Less unsaturated waters regarding calcium carbonate were observed in the upper Cuiabá, Miranda/Aquidauana and Apa rivers; $SI_{calcite}$ ranging between -3 and 0.7 (Figure 2c). More unsaturated waters were found in the São Lourenço and Negro basins.

In general, rivers in the UPB transport a high sediment concentration and total suspended solids are usually high, such as in the Taquari, Miranda, São Lourenço, and Vermelho rivers. The total suspended solids mean concentration in the tributaries reached 290.0 mg l^{-1}, higher than in the Paraguay River, where the suspended solids oscillated around 50.0 mg l^{-1} (Figure 2d). In the floodplain these values can diminish a little, such as 114.5 and 145.5 mg l^{-1}. The concentration of Chlorophyll *a* in the UPB rivers, in general, was low, with means around 1.0 to 2.0 µg l^{-1}.

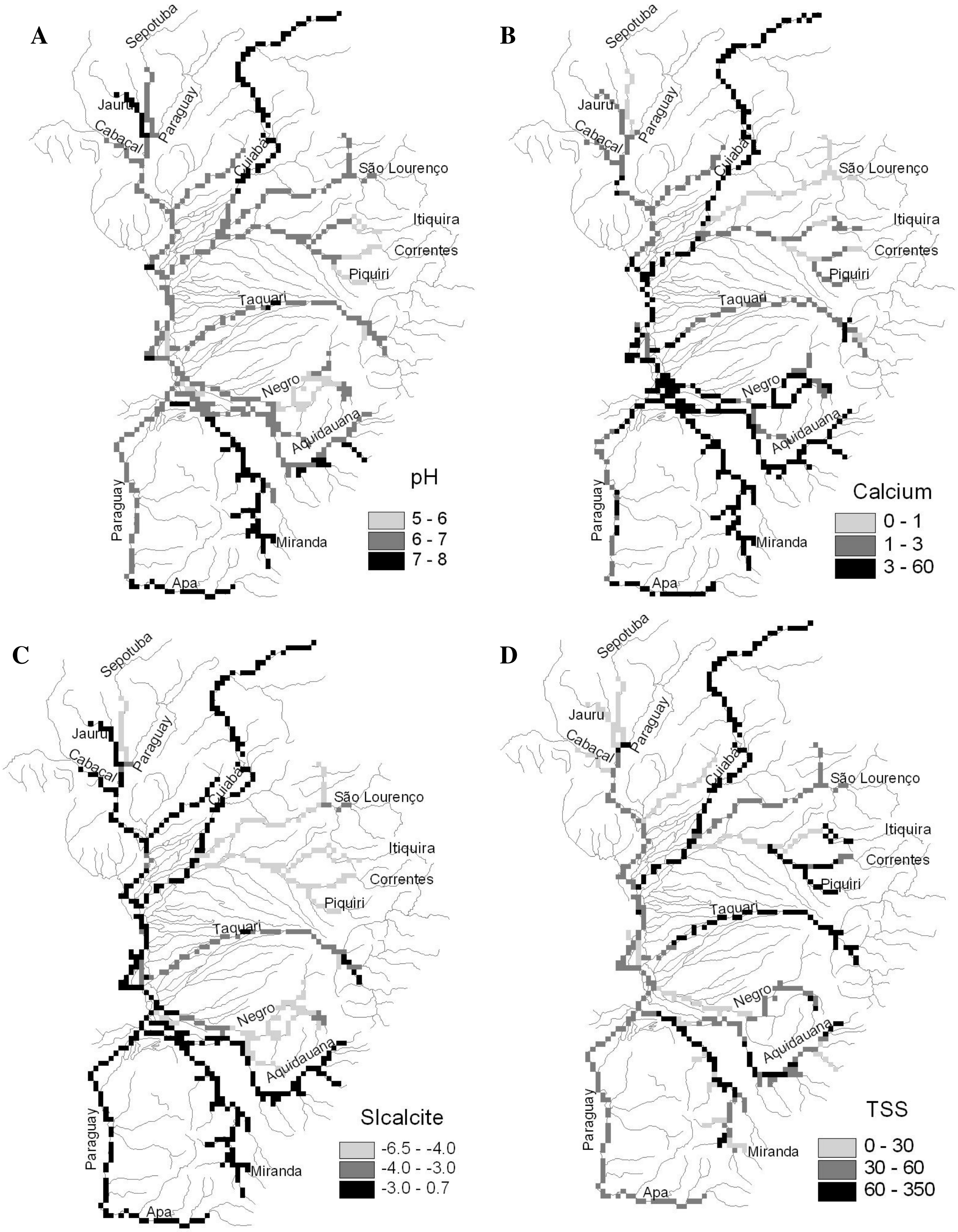

Figure 2. Spatial variation of limnological variables in the UPB. A) pH, B) calcium concentration (mg l^{-1}), C) $SI_{calcite}$, D) TSS (mg l^{-1}). Data are mean of values collected during low water phase.

Warmer water was observed in the UPB, oscillating between 16.0 and 34.5°C. Minimum temperatures were recorded between May and July and maximum 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 have minimum dissolved oxygen more than 2.0 mg l^{-1}; however it can be low as 0.0 mg l^{-1} during rising water in the lower sections, located at floodplain. Oxygen depletion events, defined as oxygen concentrations falling below 2.0 mg l^{-1}, were recorded in the Paraguay River floodplain including the lower portion of tributaries, such as Miranda and Cuiabá rivers (Figure 3). These events were observed once a year, and might last for 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 occur from February to May when temperature is around 30.0 °C, and can reach 34.0 °C, and usually stop when temperature decreases, resultant of cold fronts coming after May.

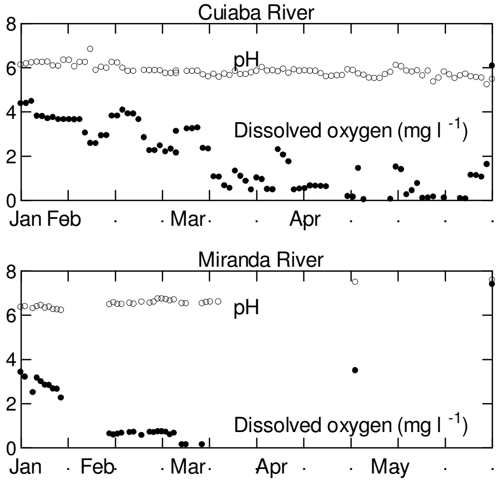

Figure 3. Short-term variation (day) of dissolved oxygen and pH during 2007 oxygen depletion events in the Cuiabá River (top graph) and Miranda River (bottom graph).

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 regions where *L. fortunei* is not recorded yet, such as Cuiabá, Negro, upper Miranda/Aquidauana and Apa. There is medium risk that the species may become established in the north of UPB, Paraguay River, Cabaçal and Jauru rivers, and in the middle of UPB, Taquari basin and Negro River tributaries. São Lourenço and Sepotuba rivers have a low invasion risk. $SI_{calcite}$ prediction showed more regions with high invasion risk than calcium concentration, mainly in the Paraguay River, Cabaçal, Jauru and Taquari rivers and with lower risk in the Negro River (Figure 4b).

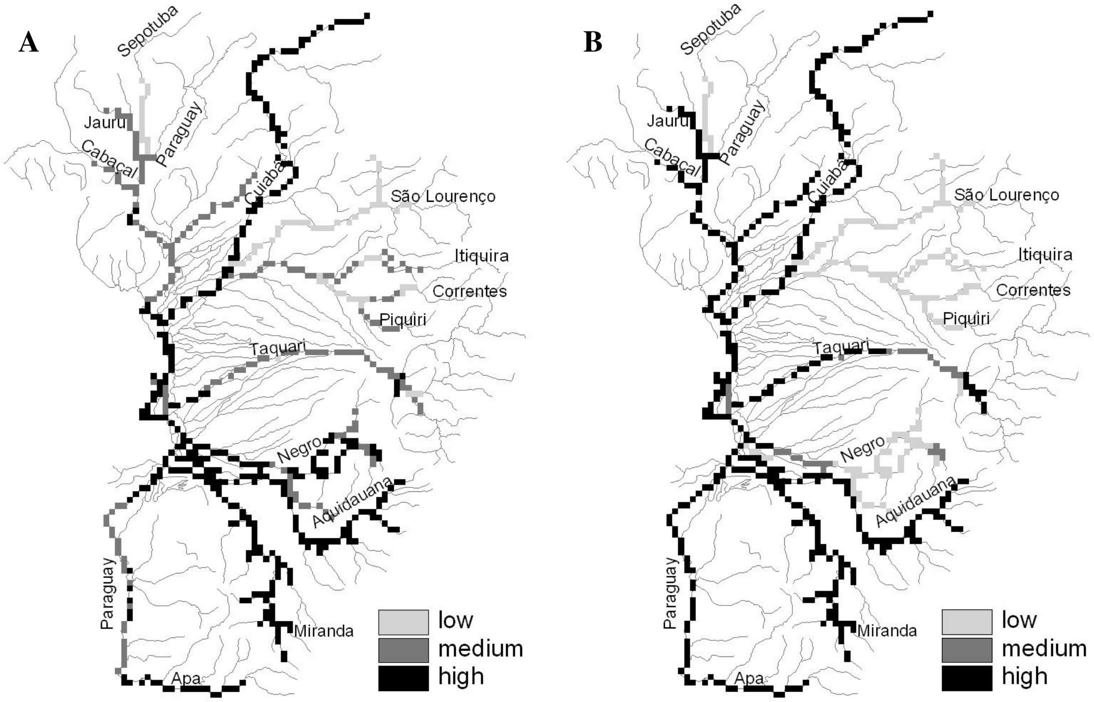

Figure 4. Potential distribution of *L. fortunei* in the UPB based on calcium concentration (A) and $SI_{calcite}$ (B) as the environmental variables. We considered low risk of *L. fortunei* occurrence in rivers where mean of calcium concentration was between 0.0 and 1.0 mg l^{-1} and $SI_{calcite}$ 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.

Results from the GARP best subset models using $SI_{calcite}$ (Figure 5a) or combined limnological variables (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 $SI_{calcite}$ empirical forecast, pointing out more risk for Cabaçal, Jauru, and portions of the upper Paraguay River, Cuiabá, and Aquidauana rivers better than the combined variables model. In general, both models predicted low risk for São Lourenço basin, Taquari, and Negro rivers.

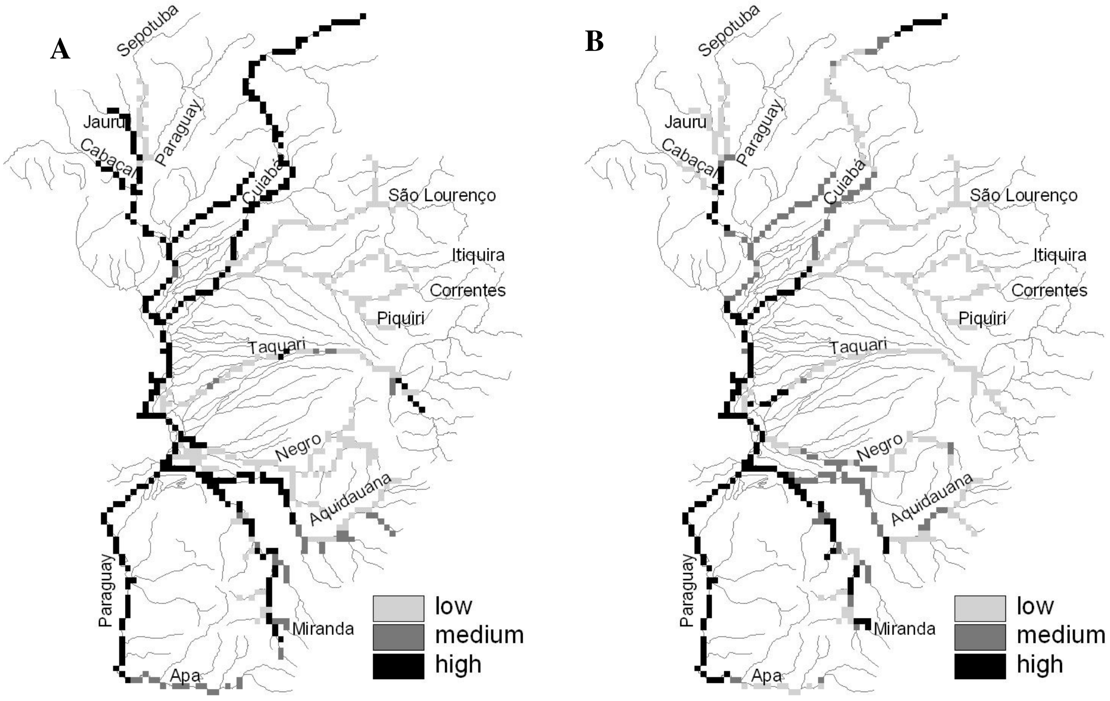

Figure 5. Predicted potential geographic distribution of the *L. fortunei* in the UPB resultant from GARP model based on the Pantanal occurrence data. (A) variable: $SI_{calcite}$; AUC= 0.75, accuracy= 95.6%, omission error= 4.3% (B) variables: calcium concentration, conductance, pH, water temperature, 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.

Maxent does not produce probabilities as a result. 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 Cuiabá, Miranda, Aquidauana and Apa rivers, upstream section of Paraguay River, Jauru and Cabaçal are the regions with better conditions for *L. fortunei* establishment. Differently from other models, the Maxent combined variables results (Figure 6b) considered São Lourenço River with good conditions to support *L. fortunei* and Maxent $SI_{calcite}$ indicated that Taquari River also has favorable conditions to *L. fortunei* colonization.

Considering results from all modeling most water in Paraguay basin have conditions to *L. fortunei* development.

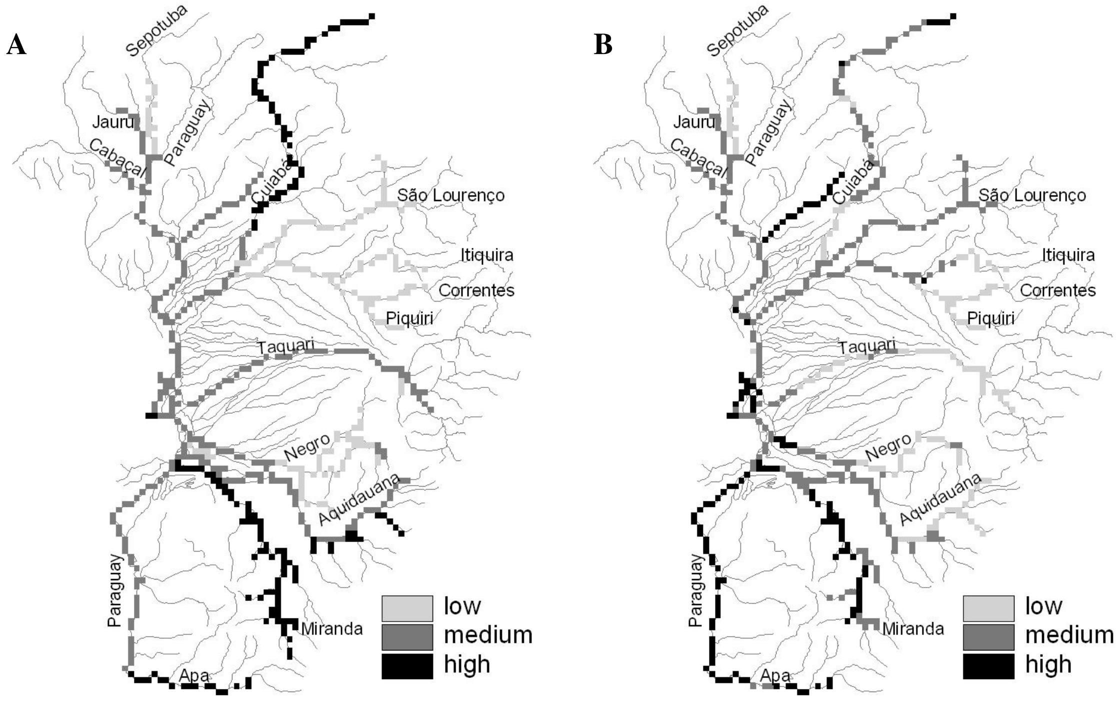

Figure 6. Predicted potential geographic distribution of *L. fortunei* in the UPB resultant from Maxent model based on the Pantanal occurrence data. (A) variable: $SI_{calcite;}$ AUC= 0.77, threshold value= 4.6; (B) variables: calcium concentration, conductance, pH, water temperature, dissolved oxygen 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.

Brazilian river systems limnological characterization

Conductance among Brazilian aquatic systems ranges from 0.0 to more than 7,000 µS cm^{-1} (Table 1). More ionic concentrated waters are in the Atlantic region (North and South) although some environments can have waters with very low conductance in these regions also. The Amazon River system also has large variation in conductance, and has the most ion dilute and acid waters. The Paraguay, Paraná, Tocantins-Araguaia, and São Francisco systems have conductance around 100.0- 200.0 µS cm^{-1}. Conductance is around 400.0 µS cm^{-1} in the Uruguay system. Values of pH between 7.0 and 8.0 are more common, but can be lower than 6.0 in the Amazon and Paraguay systems (Figure 7A).

Calcium concentration ranges from as low as 0.0 and up to 20.0 mg l^{-1} in most rivers, and exceeds 100.0 mg l^{-1} in some Atlantic system regions (Figure 7B). Waters from the Amazon, Tocantins-Araguaia and Paraguay rivers can have concentrations of less than 1.0 mg l^{-1}. All waters are closer to saturation in relation to the $CaCO_3$ than the Paraguay River, except some waters from the Amazon system, and above the minimum estimated for the *L. fortunei* establishment based on Paraguay River values ($SI_{calcite} < -4.0$) (Figure 7C). For a concentration of calcium equal to 1.0 mg l^{-1}, different values of $SI_{calcite}$ were predicted in each system: Paraguay, $SI_{calcite}$= -4.2 (r^2= 0.67, p= 0.00), Amazon= -2.6 (r^2= 0.42, p=0.00), Araguaia-Tocantins= -2.5 (r^2= 0.44, p=0.00), Paraná= -1.8 (r^2= 0.11, p=0.01), São Francisco= -1.3 (r^2= 0.27, p=0.01) and Uruguay= -0.7 (r=0.37, p=0.00), North Atlantic = -2.0 (r^2= 0.52, p= 0.00), South Atlantic = 1.5 (r^2= 0.11, p=0.00). These differences reflect variation in hydrogeochemical composition and aquatic metabolism across these river systems.

Mean water temperature is between 25 and 29 ºC. Minimum dissolved oxygen is higher than 1.9 mg l^{-1}, with means varying between 3.1 and 8.0 mg l^{-1}. Chlorophyll *a* means can be as low as 0.0 to 10.0 µg l^{-1} in the Paraguay and Sao Francisco systems, medium as 10.0 to 100.0 µg l^{-1} in the Amazon, Tocantins-Araguaia and North Atlantic, and higher than 100.0 µg l^{-1} in the Paraná and South Atlantic systems (Table 1).

Table 1. Limnological characterization of Brazilian river system. Data were provided by “Brasil das Águas Project” database except for Paraguay system.

Brazilian systems	Parameters	Water Temperature	Dissolved oxygen	Conductance	Chlorophyll a
	N	127	127	127	127
Paraguay	Min	17.1	3.1	4.2	0.0
	Max	31.1	8.6	469.2	5.9
	Mean	24.9	6.7	69.8	1.5
	SD	2.9	1.2	72.4	1.1
Amazon	*N*	421.0	365.0	421.0	419.0
	Min	23.4	3.4	0.0	0.0
	Max	36.2	8.8	1516.0	608.2
	Mean	29.1	6.8	233.2	15.9
	SD	1.7	0.8	252.4	36.4
Paraná	*N*	65.0	48.0	65.0	64.0
	Min	20.6	3.3	5.0	0.0
	Max	30.2	8.3	717.0	972.1
	Mean	25.4	6.7	173.1	163.4
	SD	2.0	1.6	196.5	266.8
S. Francisco	*N*	30.0	30.0	30.0	30.0
	Min	22.9	5.6	30.0	0.0
	Max	28.6	9.1	312.0	76.7
	Mean	26.3	7.5	112.4	5.7
	SD	1.5	1.0	95.2	14.9
Tocantins-Araguaia	*N*	95.0	95.0	95.0	95.0
	Min	23.2	6.4	0.1	0.0
	Max	30.8	9.8	2411.0	181.9
	Mean	27.8	8.1	129.9	15.1
	SD	1.7	1.2	328.3	28.6
Uruguay	*N*	29.0	29.0	29.0	29.0
	Min	24.2	1.9	493.0	3.3
	Max	31.6	8.2	611.0	634.3
	Mean	27.4	3.1	536.3	142.0
	SD	2.1	1.1	31.7	191.0
North Atlantic	*N*	113.0	113.0	107.0	113.0
	Min	24.4	4.2	0.4	0.0
	Max	36.4	9.8	7671.0	688.3
	Mean	28.3	8.1	597.7	24.9
	SD	1.7	0.9	1365.8	72.2
South Atlantic	*N*	113.0	113.0	93.0	113.0
	Min	20.8	2.5	53.0	0.0
	Max	31.3	10.3	6802.0	697.0
	Mean	26.5	8.0	561.6	110.8
	SD	2.0	1.7	1035.2	168.3

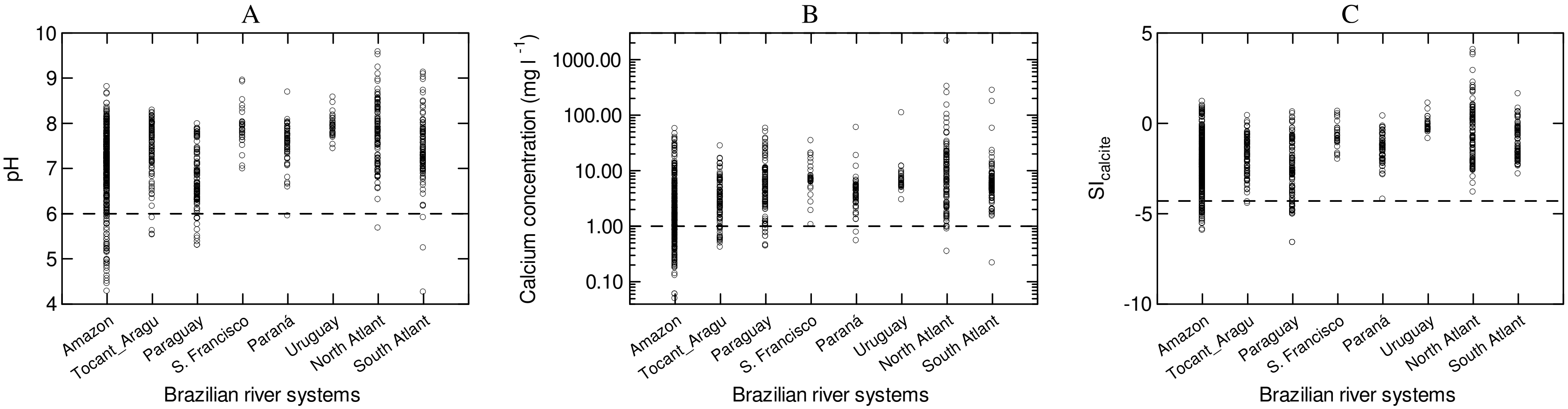

Figure 7. Ranges of pH (A), calcium concentration (B) and SI$_{calcite}$ (C) in the Brazilian river systems. Limnological data set were provided by "Brasil das Águas Project". Limits used on pH, calcium and SI$_{calcite}$ were established considering Oliveira et al. (in review) and Oliveira et al. (in preparation).

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

The same procedures used to predict *L. fortunei* in the upper Paraguay basin were performed to predict the occurrence of *L. fortunei* in a large extension of Brazilian river systems. Prediction of potential distribution of *L. fortunei* based on calcium concentration and $SI_{calcite}$ indicates that most waters in Brazil have medium to high risk of establishment (Figure 8A and 8B).

Included in the regions with high risk are the estuaries of the Amazon and Tocantins rivers, some regions in the Atlantic (North and South) such as the Mearim River estuary (Maranhão), and Jequitinhonha, Mucuri, Doce, and Paraiba do Sul rivers. Medium to low probabilities were found for some tributaries of the Amazon River, such as Negro, and portions of Tapajós and Xingu rivers, as well as Araguaia River.

Results from GARP and Maxent models using SIcalcite (Figure 9A and 10A) showed fewer regions with high risk of *L. fortunei* establishment in the Brazilian rivers than the empirical forecasting using calcium concentration and SIcalcite, however most environments presented medium to high risk of *L. fortunei* establishment. The Maxent combined variables model indicated much less regions with conditions for *L. fortunei* establishment than other models. GARP and Maxent models using $SI_{calcite}$ predicted medium risk of *L. fortunei* establishment in the Uruguay River and Guaiba Lake where the species is already present.

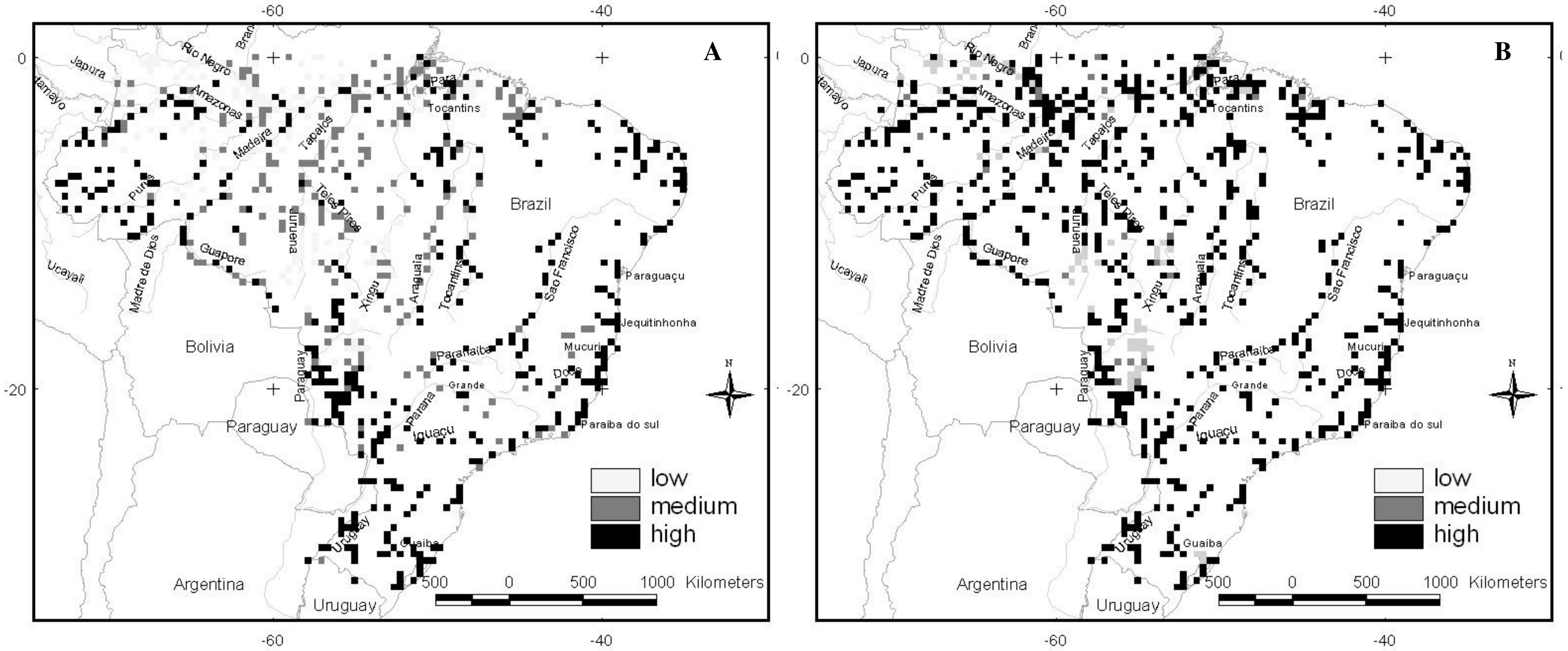

Figure 8. Potential distribution of *L. fortunei* in the Brazilian river systems using calcium concentration (A) and $SI_{calcite}$ (B) as variables. We considered low risk of *L. fortunei* occurrence in rivers where mean of calcium concentration was between 0.0 and 1.0 mg l^{-1} and $SI_{calcite}$ was < -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.

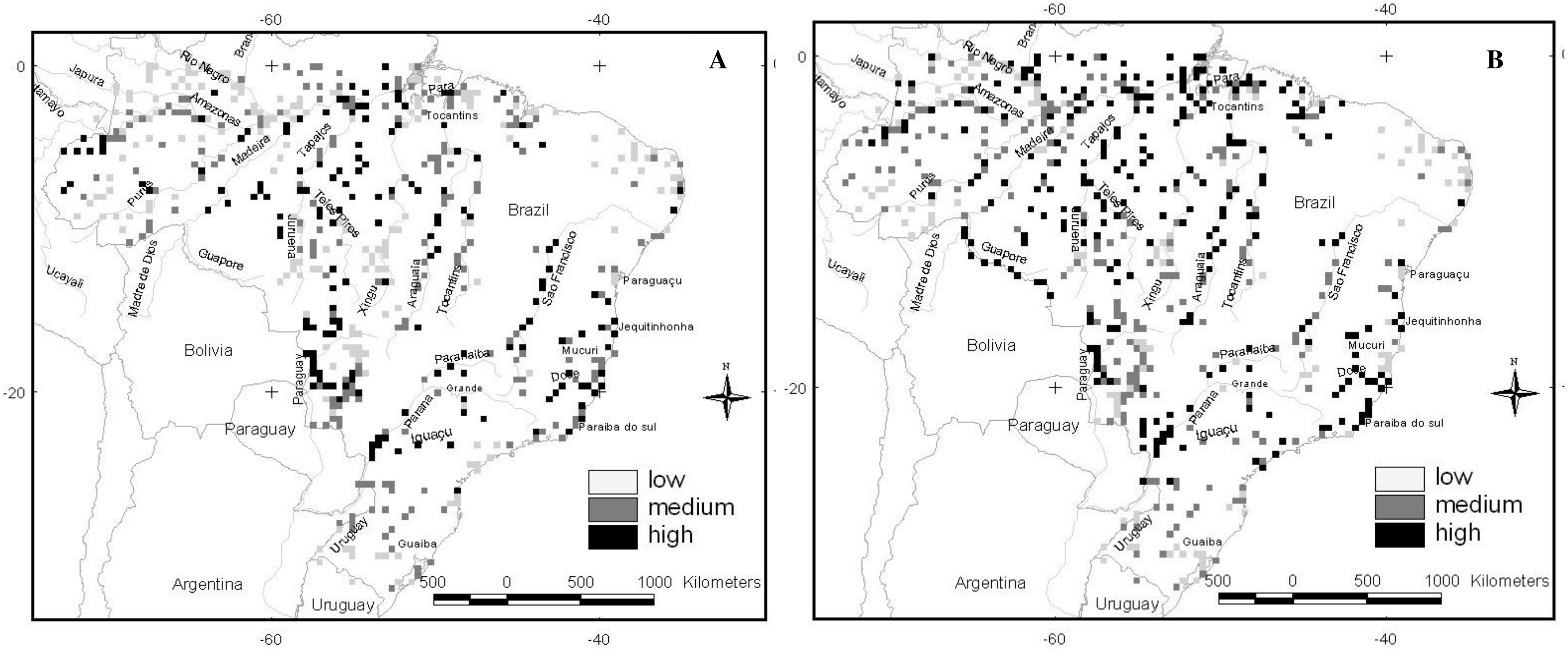

Figure 9. Predicted potential geographic distribution of *L. fortunei* in the Brazilian river systems resultant from GARP model based on the Pantanal occurrence data: (A) variable: $SI_{calcite}$, AUC= 0.82, Accuracy= 94%, Omission error= 5.8% (B) variables: calcium concentration, conductance, pH, water 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.

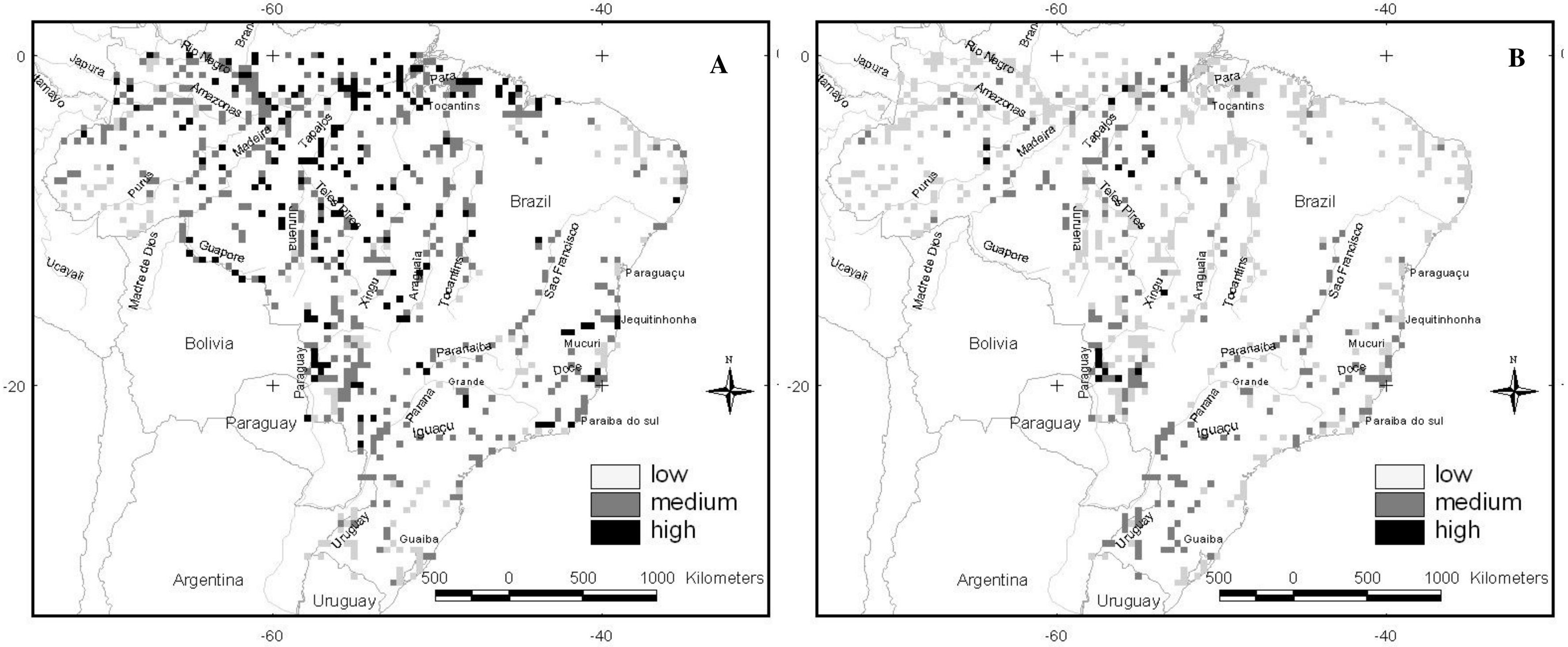

Figure 10. Predicted potential geographic distribution of the *L. fortunei* in the Brazilian river systems resultant from Maxent model based on the Pantanal wetland occurrence data: (A) variable: $SI_{calcite}$, AUC= 0.82, threshold value= 3.0 (B) variables: calcium concentration, conductance, pH, water temperature and dissolved oxygen, AUC= 0.96, threshold value= 5.5. Three colors 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).

Discussion

Expansion of *L. fortunei* in the upper Paraguay basin and other Brazilian river system

The models based on the Pantanal wetland occurrence area and limnological variables from the UPB showed a gradient of invasion risk where *L. fortunei* will be able to live in most rivers of the UPB and in the Brazilian river systems. Even though there are differences among our map outputs, the predictions indicated that the UPB has high-risk regions for the establishment of *L. fortunei*. The forecast for the rest of Brazil, predominantly those from empirical models, also showed high potential distribution for most rivers, although Maxent models showed fewer regions where *L. fortunei* could establish itself.

In most places where *L. fortunei* occurs naturally or as an invader, pH is above 6.4 and calcium concentrations are higher than 2.4 mg l^{-1} (Morton 1975; 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. in review). Considering the tolerance of *L. fortunei* to diluted water as demonstrated by Deaton (1992) we can anticipate that waters with less than 1.0 mg l^{-1} of calcium concentration have low risk of *L. fortunei* establishment, but that it is not impossible to occur. Thus, the Paraguay and other Brazilian systems have medium to high probability of invasion, and less water with low risk still have some chance to support *L. fortunei* if we consider some chances of invasion in waters with calcium less than 1.0 mg l^{-1}.

In general, 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 with calcium concentration below this threshold. The same pattern was observed in the other Brazilian systems considered here. Therefore, calcium concentration is a good predictor of the potential distribution of *L. fortunei* even in a large scale such as that of Brazilian rivers system.

$SI_{calcite}$ provides results comparable to those predicted by calcium concentration. It 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) in the studies with *D. polymorpha* in North America. The minimum $SI_{calcite}$ value observed in the Paraguay system where *L. fortunei* is present was -4.0, in water with less than 1.0 mg l^{-1} of calcium. All other

Brazilian system are closer to saturation than Paraguay system, and it is likely that *L. fortunei* would have better conditions for calcification and therefore growth in those other systems compared to the Paraguay River system. Waters with the same calcium concentration are quite different with respect to carbonate saturation, and $SI_{calcite}$ can be a better predictor for *L. fortunei* than calcium. There is lack of data on whether *L. fortunei* is able to establish itself in waters more undersaturated than the Paraguay River value ($SI_{calcite}$ <-4). During oxygen depletion events, when $SI_{calcite}$ values were around -5.5, and high mortalities were observed (Oliveira et al. in preparation).

Until now, niche models like GARP and Maxent had not employed most used limnological variables like pH and calcium to predict the spread of aquatic mollusk invasive species, although these variables have been used in other predictive studies (Ramcharan et al. 1992; Neary and Leach 1992). Neary and Leach (1992) predicted the expansion of the zebra mussel in Ontario province (Canada) using calcium and pH, and the current distribution of zebra mussel after about 16 years (Zoltak 2008) matches the high risk areas in that prediction.

Other important biotic variables such as density and chlorophyll *a* and abiotic such as the kind of substratum, water flow and introduction routes could not be incorporated in the models because they were not available for most sites, or were not reliable enough. There is no standardized information on suspended solids in Brazilian waters, so we were not able to use this variable to predict *L. fortunei* expansion in Brazilian rivers.

The effects of turbidity were observed in zebra mussel population in large rivers like the Mississippi River (Alexander et al. 1994; Allen et al. 1999). The effects were observed when combined with high temperature, the most important factor limiting zebra mussel in the lower Mississippi River. Although there is no information about the effects of suspended sediments in *L. fortunei*, we have discussed the combination of high concentration of suspended sediments, low food availability and high water velocity as a limiting factor in the Miranda River (UPB), especially to larval settlement (Oliveira et al. in review, Oliveira et al. in preparation).

In the UPB, Cuiabá, Miranda/Aquidauana and Apa rivers are the systems with highest potential of *L. fortunei* occurrence, however, these rivers present relatively high values of total suspended solids and low chlorophyll *a*, close to 0.0 $\mu g\ l^{-1}$, which can render these regions less susceptible to invasion, compared to waters from other rivers that have lower suspended sediments concentration.

Chlorophyll *a* usually can be low, as in the Paraguay River, 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). Most water in other Brazilian systems has chlorophyll *a* higher than in the UPB; it should therefore not be a factor limiting the occurrence of *L. fortunei*.

Prediction from models was robust since all models predicted medium to high probability in the Paraguay River and most of them predicted occurrence in the Paraná River in the sites where the species is already present. However, both GARP and Maxent models failed to predict *L. fortunei* expansion in several relatively large-scale areas (Brazilian systems) where calcium concentration and $SI_{calcite}$ values are favorable to its establishment.

Although there were no remarkable differences, $SI_{calcite}$ model results are closer to calcium concentration predictions if compared to models based on multiple variables, maybe because $SI_{calcite}$ data is plotted in a logarithm scale, and variability between maximum and minimum was reduced. Especially in the other Brazilian systems the range of variables such as conductance and calcium concentration were very wide compared to the occurrence area in the Pantanal, and some of these regions present low risk by GARP and Maxent but have medium to high risk when calcium and $SI_{calcite}$ empirical models were used. This fact indicates that the algorithm model might not be trusted when the values surpass the ranges of the occurrence area, used by the model to build the ecological niche.

There are many other uncertainties about ecological niche modeling; accuracy is dependent on the sample size, or the quantity of data needed to discover the correct model (Stockwell and Peterson 2002). Regardless of the sample size, choosing variables to use is also difficult. Several simulations were conduced and different results were obtained for each combination of variables in this study. So, we decided to not control or influence the result inserting or taking variables. We used all available and reliable variables.

The larger the spatial scale, the more the uncertainties; thus there are more uncertainties regarding Brazilian rivers than in UPB. Projection maps using grids less than 0.4 pixels are difficult to represent in the large scale of Brazilian rivers system; on the other hand, grids with 0.4 pixels arranged several sample points close to each other, contributing to erroneous outputs. Interpolation points are one way to minimize these effects, as was done in the UPB case. However, it may not work satisfactorily when applied to large areas as Brazil. Some adjustments in this methodology need to be made for better results. The forecast of *L. fortunei* establishment in Brazilian rivers using calcium distribution and ecological niche modeling based on limnological variables is the first attempt to know the potential of establishment of this species in Brazilian waters.

Our predictions for the establishment of the golden mussel in Brazilian rivers are consistent with analyses conducted by Kluza and McNyset (2005) in large spatial scale modeling the species expansion throughout the Americas. According Kluza's model *L. fortunei* will expand to a large area in Brazil and high risk regions include lower Amazon River, but not the estuary zone, and lower section of Tapajós, Xingu, and parts of Guaporé River. Lower Araguaia-Tocantins, above estuaries section, Paraguay-Paraná and Uruguay systems are in the region with high risk also. Some Atlantic regions (North and South) are also under high risk of *L. fortunei* introduction. Rivers located in the upper and middle Amazon River, Negro River, middle and upper sections of Tapajós and Araguaia–Tocantins have less chance of *L. fortunei* occurrence according to Kluza's model. The São Francisco basin and portions of the Paraná basin such as the Paranaíba River are also included in the low-risk group.

According to our predictions important sites located in the Amazon estuary as well as waters in the upper section of Amazon tributaries, that are close to the Paraguay system, such as Tapajós, Teles Pires, Araguaia, and others rivers in the Amazon basin have limnological conditions suitable for the occurrence of *L. fortunei.* However, they were predicted as low-risk areas by Kluza's model. Low risk was also predicted for the Sao Francisco system and parts of the Paraná system, such as the Paranaíba River, in Kluza's model, contrasting with our prediction that these regions have medium to high potential of *L. fortunei* invasion. Environmental variables and spatial scale are likely responsible for these differences between Kluza's model results and our results. Kluza's model was built using East Asian occurrence data and climatological and topographic variables. The advantage of our method based on limnological variables is that it provides more detailed and specific information for aquatic systems; we have chance to analyze each river system separately, and for more details a small scale can be used, as we did in UPB.

We believe that *L. fortunei* has the means to spread and that propagule will reach most Brazilian rivers in the future, as occurred with *Corbicula fluminea* (Muller 1774), in the South America, particularly in Brazil. *C. fluminea* is another invasive species native to the same environments from *L. fortunei*. The current distribution of *C. fluminea* might be a good indicator of the future distribution as *L. fortunei* in Brazil, since co-occurrence has been observed (Morton 1996).

The full distribution of *C. fluminea* in South America is not well known, but some records have shown *C. fluminea* in the Amazon River estuary and Tocantins River (Beasley et al. 2003; Mansur et al. 2004; Pimpão and Martins 2008), Paraná system (Suriane et al.2007), and Uruguay River (Mansur et al.2004), Cuiabá and Paraguay in UPB, and Araguaia River (Callil et al. 2008). Callil and Mansur (2002) verified that *C. fluminea* do not attain high densities in the Pantanal such as in other places in South America, and associated to the recent introduction and tolerance to environmental variables. More information on this species will help define the environmental limits for *L. fortunei* and thus improve the quality of its distribution models. Additionally, we will be able to confirm our predictions where *L. fortunei* is most successful when more regions have been invaded.

Other factors affecting the expansion of *L. fortunei*

Considering the high potential risk of the entirety of Brazilian freshwaters for the establishment of *L. fortunei*, management of invasions should focus on potential points of entry into these regions. According to Boltovskoy et al. (2006), the major South American hydrographic basins like Amazon, Orinoco, and Magdalena are readily accessible through their estuaries by ocean-going vessels, which makes them highly vulnerable to invasions via ships ballast water. In central Brazil transport of mussels by recreational boaters and other human activities will also contribute to the expansion of *L. fortunei*. Regions with high potential to invasion in the Paraguay and Paraná systems are very close to those regions with high in the Amazon and Sao Francisco basins.

Rapid spread of *L. fortunei* in the Paraguay-Paraná system might be attributed to biological characteristics such as high reproductive rate and free swimming larval stages and, specially, to the means of transport associated with human activities such as in the Paraguay-Paraná waterway. Attachment to vessels is the most important dispersal mechanism of *L. fortunei* in South America. The expansion of *L. fortunei* in the Paraguay River was fast in the main channel but it was slow between Paraguay River and tributaries, where boat traffic is lower, delaying the expansion of *L. fortunei* in the UPB. Technical reports from Oliveira et al. (2003) proposed some measures to reduce the dispersion of *L. fortunei* including sanitary barriers and use of antifouling paints in the embarkations, the main way to prevent dispersal of this species between the Paraguay River and its tributaries.

Some floodplain areas, such as the lower Cuiabá River, were also ascribed high risk based on the GARP and Maxent approaches. This said, although these areas have a good interaction with the Paraguay River by navigation *L. fortunei* has not been recorded there after nine years of invasion. Only 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, namely oxygen depletion events, because they restrict the occurrence of *L. fortunei* once a year.

During much of the year dissolved O_2 concentrations in rivers within the Pantanal wetland are often around 50-60% of atmospheric equilibrium, but can be below 2.0 mg l^{-1}, especially during rising waters. Oxygen depletion events in the Pantanal are associated with simultaneous stress factors including not only low or even absence of O_2 but also higher temperature, lower pH, and higher free CO_2 (Calheiros and Hamilton 1998; Hamilton et al. 1997).

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. 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 to dissolved oxygen events also, mainly because it occurs during the reproductive period (Oliveira et al. in preparation).

Water level fluctuation in the Paraguay River is about 5m and represents another limiting factor for *L. fortunei.* It is possible that a sequence of stressful factors, such as low water levels that kills 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.

Beyond the floodplain area these events do not occur and *L. fortunei* can reach high densities in regions predicted to be of high risk of invasion such as Cuiabá and Miranda/Aquidauana Rivers. These rivers are the ones most visited by tourists. Fishing activities usually include visiting more than one river during the same trip, and are therefore an important factor enhancing transference of the species from one place to another.

Another anthropic factor enhancing the invasion of the golden mussel is dam construction. According to Johnson et al. (2008) impoundments lakes in the Laurentian Great Lakes region present 2.4 to 300 times more chances to have non-indigenous species, and the impoundments frequently support multiple invaders. Clear waters with available food are good conditions for high density population of *L. fortunei* in the Paraná power plant reservoirs, Brazil. In the UPB, existing power plants as in the Manso River (tributary of Cuiabá River) and small others in the Jauru, Piquiri, Sepotuba, and Correntes rivers will increase the potential of *L. fortunei* invasion, even in the small ones which did not show great vulnerability to invasion according to our prediction. The Brazilian government has plans to install several other small hydroelectric plants (< 30MW) in the UPB, and this will increase the chances of *L. fortunei* dispersion into this basin.

Considerações finais

Neste estudo corroboramos a idéia de que *L. fortunei* tem ampla tolerância a fatores ambientais e sugerimos ampliar os limites inferiores daqueles propostos na literatura, baseado nas condições do Pantanal sul-matogrossense. Esses valores são pH > 6.0, cálcio > 1.0 mg l^{-1} e $IS_{calcita}$ > -4.0. Abaixo desses valores consideramos que *L. fortunei* terá menos chance de se estabelecer.

Consideramos o cálcio uma boa variável para prever a distribuição potencial de *L. fortunei* e propusemos um novo índice, $IS_{calcita}$, baseado na saturação do $CaCO_3$. Esses parâmetros são fáceis de utilizar comparados a modelos matemáticos,

Resultados baseados na modelagem utilizando variáveis limnológicas indicaram que *L. fortunei* poderá se expandir para os principais rios da bacia do Alto Paraguai e para os principais rios do Brasil, caso seja transportadas para essas áreas. No entanto, não significa que *L. fortunei* vá se expandir para todo o Brasil, pois uma série de fatores ligados ao transporte e limitação do estabelecimento característico de cada região poderá ser predominante em relação às características da água.

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