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Investigação sobre o status de invasão da espécie exótica invasora *Procambarus clarkii* Girard, 1852 (Crustacea, Decapoda, Cambaridae) no Brasil

Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Mestre em Biologia Animal.

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Dissertação aprovada em ______ de ____________ de _______.

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Sumário

Prefácio

O deslocamento de espécies na superfície da Terra, seus padrões de ocupação e distribuição estão intimamente ligados à história da vida em nosso planeta, moldados por um equilíbrio dinâmico entre migrações e extinções. Apesar disso, nos últimos séculos, especialmente a partir da época das grandes navegações, o homem tem alterado de uma forma sem precedentes a distribuição de plantas, animais e microrganismos no planeta, estabelecendo uma nova dinâmica biogeográfica na qual o transporte de espécies se dá de formas muito mais eficientes e percorrendo distâncias muito mais longas do que ocorreria em circunstâncias não favorecidas pela tecnologia humana.

Este transporte favorecido pelo homem é, na maioria das vezes, proposital e visa assegurar a segurança alimentar ou a disponibilidade de outros recursos necessários à nossa sobrevivência e demais atividades humanas; no entanto há também aquelas espécies que viajam despercebidas, "na carona" dos itens que estão sendo movidos propositadamente, como as espécies transportadas, por exemplo, com a água de lastro dos navios. Independente da forma de introdução e do vetor de transporte, o fato é que na maioria dos países, o estabelecimento de espécies exóticas é ignorado ou quando conhecido, poucas são as medidas realizadas para controlar e/ou mitigar os problemas acarretados pelas mesmas. Um dos países em que o problema da invasão biológica vem sendo praticamente ignorado é o Brasil, sendo que este detém mais de 15% de todas as espécies de plantas e animais do planeta, assim como muitos dos ambientes mais conservados. Isso faz com que a preocupação com esta temática seja urgente. Nosso país busca o progresso e procura garantir emprego e acesso à saúde, educação e saneamento para todos, e isso só poderá ser alcançado com investimento em programas de desenvolvimento. Estes programas, todavia, devem ser planejados e desenvolvidos com cautela e baseados em estudos de impacto, para evitar problemas posteriores como a facilitação de eventos de invasão pelo incentivo de cultivo de espécies exóticas, por exemplo.

Considerando que a erradicação de espécies não nativas já estabelecidas é muito difícil e dispendiosa, a melhor abordagem é a prevenção, e para isso, a utilização de protocolos de análise de risco que incluam parâmetros ambientais é uma das medidas que vem se mostrando muito eficiente. A conscientização pública e o investimento em educação ambiental também são estratégias muito úteis e eficazes.

Este trabalho foi um primeiro passo para compreender o processo de invasão do lagostim vermelho no Brasil, assim como trazer à atenção de todos, a amplitude dos problemas que podem ser gerados com a dispersão desta espécie em território nacional. É importante também ter a clareza de que o controle deste processo só poderá ocorrer através de cooperação pública em todos os níveis. Precisamos que as pessoas deixem de vender, comprar e criar estes animais, o que é considerado ilegal pela constituição brasileira.

O conteúdo deste estudo está organizado em quatro capítulos: o primeiro traz a introdução geral da dissertação, expondo de forma ampla a problemática de pesquisa; o segundo capítulo abrange o manuscrito “The red swamp crayfish: Biology, ecology and invasion- a brief review”; o terceiro capítulo traz o manuscrito “Distribution, introduction pathway and invasion risk analysis of the North American crayfish *Procambarus clarkii* (Crustacea, Decapoda, Cambaridae) in Southeast Brasil”; e o quarto e último capítulo traz as considerações finais do trabalho e perspectivas futuras. Cada um dos manuscritos está formatado, inclusive as referências, de acordo com as revistas para as quais serão enviados. A introdução geral e as considerações finais encontram-se formatadas nas regras da Associação Brasileira de Normas Técnicas (ABNT).

Resumo

O Brasil é um país megadiverso, entretanto a fauna e a flora encontram-se severamente ameaçadas por inúmeros fatores. Dentre eles, a introdução de espécies exóticas invasoras que, especialmente nos ambientes límnicos, é uma das grandes ameaças à conservação da biodiversidade, principalmente devido à predação, competição e introdução de novas doenças. Dentre os animais com grande potencial de invasão, casos envolvendo crustáceos são bastante documentados na literatura, como por exemplo, o lagostim Norte-Americano *Procambarus clarkii*, que é utilizado para fins de aquarismo e aquacultura em diversas partes do mundo. As informações sobre a ocorrência desta espécie no Brasil são escassas, havendo apenas cinco registros no estado de São Paulo. Inúmeros impactos já foram associados à presença desta espécie em várias partes do mundo, desde danos ao ecossistema até impactos sobre a biota nativa. Dessa forma, a dispersão de *P. clarkii* em território brasileiro pode gerar efeitos desastrosos aos ambientes límnicos. Sendo assim, o presente trabalho efetuou uma ampla revisão bibliográfica referente às características desta espécie e sua problemática de invasão no mundo, assim como investigou de forma integrada o presente, passado e futuro de seu processo de invasão no Brasil. Observou-se que o principal fator de introdução deste crustáceo no Brasil é o comércio ilegal para aquacultura e onze novas populações foram encontradas no estado de São Paulo. Adicionalmente, realizou-se uma análise de risco de invasão da espécie e constatou-se que a mesma apresenta alto poder de estabelecimento em território nacional e grande capacidade de geração de impacto. Os resultados deste trabalho alertam para a necessidade de investigações mais aprofundadas sobre o processo de invasão na América do Sul. Torna-se também imprescindível o investimento em fiscalização e em educação pública, a fim de evitar que os animais continuem sendo vendidos ilegalmente e introduzidos na natureza.

Contexto

A presente pesquisa está inserida dentro de um projeto maior intitulado: "Ecossistemas límnicos continentais e conservação dos lagostins de água doce do gênero *Parastacus* (Crustacea, Decapoda, Parastacidae) no sul do Brasil". Em linhas gerais, esse projeto objetiva formar uma base sólida de dados ecológicos e genéticos que permitam avaliar de forma correta o *status* de conservação das espécies de lagostins de água doce e, consequentemente auxiliarão no delineamento de estratégias de conservação. Dentre os dados importantes que deverão ser levantados, estão aqueles referentes à presença da espécie exótica invasora de lagostim de água doce *Procambarus clarkii* Girard, 1852 (Crustacea, Decapoda, Cambaridae) no Brasil. Esta espécie consiste, sabidamente, em um grande risco para as espécies nativas e para os ambientes nos quais for introduzida. Dentro dessa questão específica relacionada à *P. clarkii*, estão sendo desenvolvidos os seguintes projetos: (1) as interações comportamentais entre *P. clarkii* e as espécies de lagostins nativos, (2) infecção das populações de *P. clarkii* com o fungo *Aphanomyces astaci* Schikora, 1906 e a possível transmissão para as espécies nativas e (3) conhecimento da dinâmica de invasão, distribuição e potencial de impacto das populações de *P. clarkii* nos ambientes límnicos brasileiros.

-Capítulo I-

Introdução Geral

Introdução Geral

A biodiversidade brasileira se destaca quando comparada com quase todas as outras regiões do planeta. A combinação de sua grande extensão territorial, somada à sua diversidade geográfica e climática, contribui para que o Brasil abrigue entre 15% e 20% das 1,5 milhão de espécies descritas na Terra (LEWINSOHN E PRADO, 2002). Esta considerável biodiversidade vem enfrentando inúmeras ameaças devido à deterioração da qualidade ambiental dos ecossistemas aquáticos e terrestres, resultado de atividades humanas como incêndios e queimadas, fragmentação e perda de habitat, introdução de espécies invasoras, poluição e mudanças climáticas (BRASIL, 2010). Estes fatores acabam levando à redução populacional de algumas espécies e/ou aumento da abundância de outras, além de favorecer o estabelecimento de espécies exóticas (HADDAD E PRADO, 2005).

Dentre os ecossistemas brasileiros ameaçados, os ambientes aquáticos continentais merecem especial atenção uma vez que são reservatórios de água doce, um recurso natural limitado e essencial para a manutenção da vida e para a realização de inúmeras atividades econômicas. Além disso, os ambientes límnicos abrigam uma grande diversidade de flora e fauna e possuem alta produtividade (BARTRAM E BALANCE, 1996; MITSCH E GOSSELINK, 2000). No Brasil, os fatores mais fortemente associados à degradação destes ecossistemas são a canalização de cursos d’água, a descarga de efluentes urbanos e industriais e a introdução de espécies exóticas invasoras (BRASIL, 2010).

Alguns estudos, como o realizado por RICCIARDI E RASMUSSEN (1999), sugerem que, em escalas globais, a biodiversidade nos ecossistemas límnicos tem diminuído de forma muito mais acelerada do que o observado na maioria dos ecossistemas terrestres e, à semelhança do observado no Brasil, uma das principais causas é a introdução proposital ou acidental de espécies não nativas (ou exóticas). Os ecossistemas límnicos são extremamente suscetíveis ao estabelecimento de espécies não nativas devido à forte relação entre os seres humanos e a água como, por exemplo, para transporte, comércio ou recreação, e pela alta capacidade de dispersão das espécies aquáticas (LODGE et al., 1998; BEISEL, 2001; GHERARDI, 2006).

Alguns termos utilizados em biologia de invasão são ainda muito discutidos e controversos, não havendo concenço sobre algumas definições, o que torna importante a determinação de alguns aspectos conceituais dos termos utilizados neste estudo. Foram utilizadas as definições presentes em FALK-PETERSEN et al. (2006) e LOPES (2009), onde espécie nativa (ou indígena) é aquela que vive em sua região de origem (área natural); espécie exótica (ou não-indígena) é aquela registrada fora de sua área de distribuição original passada ou presente; espécie introduzida é aquela exótica translocada, intencionalmente ou não, em função da realização de atividades humanas; espécie estabelecida é aquela exótica que possui ciclo de vida completo na natureza e indícios de aumento populacional ao longo do tempo e espécie invasora é aquela exótica estabelecida que aumenta sua área de ocupação e afeta negativamente as espécies nativas ou causa impactos a atividades socioeconômicas ou a saúde humana.

As espécies exóticas invasoras possuem a capacidade de gerar enormes prejuízos à economia, à biodiversidade e à saúde humana devido ao seu grande potencial de modificar os sistemas naturais (WALKER E STEFFEN, 1997; WILCOVE et al., 1998) e podem alterar processos ecológicos importantes como o ciclo da água, de energia e/ou de nutrientes (VITOUSEK, 1990), de forma que sua introdução em áreas não nativas pode gerar extinção local e substituição de espécies autóctones, substituição de espécies e outros danos irreparáveis aos ambientes e à biodiversidade, afetando inclusive os serviços da natureza (serviços prestados pelos ecossistemas) (CROOKS, 1998; CHORNESKY E RANDALL, 2003). Em adição, o estabelecimento de espécies alóctones pode propiciar a geração de híbridos com capacidade de deslocar ou extinguir as espécies nativas (HUXEL, 1999; CLEELAND E MOONEY, 2001).

Com relação aos impactos econômicos, podem-se ressaltar tanto danos com custo direto quanto indireto. Impactos com custo direto são principalmente aqueles relacionados aos gastos com o controle e a erradicação das espécies invasoras, como despesas com pesticidas por exemplo, ou a diminuição do rendimento de setores produção como a agricultura e a pecuária; impactos com custo indireto estão geralmente associados à perda de serviços da natureza, como a redução do suprimento de água, por exemplo. Não se sabe ainda qual o custo geral das invasões biológicas, entretanto, PIMENTEL et al. (2001) estimaram que o gasto anual associado ao problema da invasão biológica nos países estudados (Estados Unidos, Inglaterra, Austrália, Índia, África do Sul e Brasil) ultrapassa os US$ 336 bilhões. Todavia, é praticamente impossível aferir um valor financeiro que quantifique a extinção local ou total de uma espécie ou outros danos gerados à biodiversidade, de forma que o valor supracitado é uma subestimativa.

A invasão biológica também pode oferecer riscos à saúde humana quando as espécies exóticas disseminam doenças, micro-organismos ou parasitos que podem afetar o ser humano. Agentes causadores de doenças infecciosas são com frequência espécies exóticas invasoras, transmitidos aos seres humanos por animais ou importados inadvertidamente por viajantes. Estes micro-organismos podem ter efeitos devastadores sobre populações humanas já que, em região alóctone, estão longe dos seus fatores naturais de controle.

Na revisão realizada por PIMENTEL et al. (2001), os autores estimaram que o Brasil possui 11.709 espécies invasoras, entre plantas, mamíferos, pássaros e peixes de água doce, sendo que sobre os demais grupos não há nem mesmo uma ideia aproximada. A invasão biológica é a segunda maior causa de perda da biodiversidade no Brasil de acordo com o Ministério do Meio Ambiente (MMA)[1] e segundo o Relatório Nacional sobre Espécies Exóticas Invasoras publicado em 2005, foram registradas 1.593 ocorrências de espécies invasoras nos ambientes aquáticos continentais brasileiros, com a presença de 180 organismos exóticos dos quais 167 foram identificados ao nível de espécie: 116 peixes (incluindo híbridos), 19 microrganismos (incluindo microcrustáceos), 14 macrófitas (incluindo uma híbrida), seis crustáceos, quatro anfíbios, cinco moluscos, dois répteis e um anelídeo hirudíneo (MMA, 2005). Dentre as espécies que iniciaram processos de invasão no Brasil, cerca de 73% foram introduzidas de forma voluntária por questões econômicas, incluindo-se a comercialização de espécies ornamentais e animais de estimação (MMA, 2005).

Dentre as espécies exóticas invasoras dos ecossistemas límnicos, o lagostim *Procambarus clarkii* Girard, 1852 é um dos casos de grande preocupação em diversas partes do mundo devido ao seu potencial de impacto nos ambientes invadidos (ANGELER et al., 2001; SCHLEIFSTEIN E FEDELI, 2003; GEIGER et al., 2005; RODRÍGUEZ et al., 2005; GHERARDI E ACQUISTAPACE, 2007; PINTOR et al., 2008).

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[1] Disponível em: <http:// www.mma.gov.br/invasoras> Acesso em: 12 de fevereiro de 2013

Esta espécie é nativa da região Centro-sul dos Estados Unidos e Nordeste do México (HOBBS et al., 1989; HENTTONEN E HUNER, 1999; BOETS et al., 2009) e vem sendo introduzida em muitos países da Europa, Ásia, América Central e América do Sul (BARBARESI et al, 2007; GHERARDI E ACQUISTAPACE, 2007; WISÉN et al., 2008) para aquacultura, como isca para pesca e para o aquarismo (HENTTONEN E HUNER, 1999). No Quênia estes animais também foram utilizados em programas de controle biológico, a fim de reduzir o número de gastrópodos que atuavam como hospedeiros intermediários do platelminto parasito do gênero *Schistosoma*, causador da esquistossomose ou bilharíase em seres humanos (LODGE et al., 2000; APPLETON et al., 2004).

Procambarus clarkii se destaca entre os crustáceos decápodos por sua alta plasticidade ecológica e notável tolerância a modificações bióticas e abióticas (HOBBS et al., 1989; SIESA et al., 2011), o que lhe garante uma rápida adaptação a diferentes condições ambientais (CRUZ E REBELO, 2007; GHERARDI E PANOV, 2009). Isso explica a alta capacidade de estabelecimento da espécie em diferentes ambientes límnicos, desde açudes, lagos e rios até áreas inundáveis para agricultura, canais de irrigação e reservatórios (OLIVEIRA E FABIÃO, 1998; HENTTONEN E HUNER, 1999; ANASTÁCIO et al., 2009).

O período reprodutivo, assim como o recrutamento e a maturidade sexual variam de acordo com o ciclo hidrológico e outras características ambientais como a temperatura e a pluviosidade (SOMMER, 1984; GUTIÉRREZ-YURRITA E MONTES, 1999), de forma que a espécie consegue adequar sua biologia aos locais onde se estabelece e assim, garante um alto sucesso reprodutivo. O número de ovos também é bastante variável e também está relacionado a características ambientais, assim como ao tamanho da fêmea, todavia, o mais comum são proles com cerca de 300 ovos (ALCORLO et al., 2008).

Este lagostim é uma espécie onívora generalista cuja dieta oportunista favorece o seu estabelecimento em diferentes tipos de corpos d'água (GUTIÉRREZ-YURRITA et al., 1999). Podem alimentar-se de detritos animais e vegetais, macrófitas, moluscos, insetos, anelídeos, platelmintos, girinos e alevinos (HUNER E BARR, 1991; ILHÉU E BERNARDO, 1993; ILHÉU E BERNARDO, 1995; GUTIÉRREZ-YURRITA et al., 1998; GUTIERREZ-YURRITA et al. 1999; PARKYN et al., 2001; CORREIA, 2002;

BUCK et al., 2003; CRUZ E REBELO, 2005), sendo capazes de modificar substancialmente a estrutura trófica dos locais invadidos, interagindo com diferentes níveis tróficos e alterando completamente o funcionamento do ecossistema (ANGELER et al., 2001; DORN E WOJDAK, 2004; GHERARDI E ACQUISTAPACE, 2007; CRUZ et al., 2008). Com relação aos seus predadores, cita-se na bibliografia que servem como fonte de alimento para peixes, répteis, pássaros e mamíferos (DELIBES E ADRIÁN, 1987; HOLDICH E LOWERY, 1988); os juvenis também podem ser predados por ninfas de odonata, larvas de coleópteros e hemípteros aquáticos (GYDEMO E NISSLING, 1990).

Além dos atributos bioecológicos supracitados, *P. clarkii* apresenta uma notável capacidade de dispersão (BARBARESI E GHERARDI, 2000; GHERARDI et al., 2002; PAYETTE E MCGAW, 2003; GHERARDI, 2006; CRUZ E REBELO, 2007; OLDEN, 2007) e comportamento agressivo pronunciado, fato constantemente relacionado ao deslocamento de espécies nativas de lagostins de água doce em função da competição por recursos (GHERARDI E CIONI, 2004).

Diferentes autores também associam o estabelecimento desta espécie à bioturbação, aumento da profundidade e da vazão de cursos d’água, degradação da qualidade da água e alterações nas características sedimentares devido ao seu comportamento escavador (ANGELER et al., 2001; CRUZ E REBELO, 2007). Estas alterações ambientais acabam modificando todo o ecossistema aquático colonizado, suas funções e seus estados de equilíbrio, de forma que ANGELER et al. (2001) e GEIGER et al. (2005) associam até mesmo a indução de florações de cianobactérias à presença de *P. clarkii*.

Outra questão associada ao impacto de *P. clarkii*, principalmente sobre a fauna nativa de lagostins de água doce, é a presença do fungo *Aphanomyces astaci* Schikora, 1906 (Oomycetes, Saprolegniales, Leptolegniaceae) ao qual a maioria das espécies é mais suscetível (SOUTY-GROSSET et al., 2006; GHERARDI E PANOV, 2009; CAMMÀ et al., 2010; AQUILONI et al., 2011; LONGSHAW, 2011).

Além dos danos ecológicos gerados aos ambientes invadidos, *P. clarkii* é capaz de gerar impactos econômicos consideráveis, tanto de forma direta através dos gastos com medidas de manejo, controle e mitigação de danos, quanto indiretamente, já que pode afetar setores como a agricultura e a pesca (KETTUNEN et al., 2008). Os danos à agricultura estão associados às culturas que dependem de áreas alagadas, como o cultivo de arroz, onde a espécie pode comprometer os sistemas de drenagem em função de seu comportamento escavador (SOMMER, 1984; GAUDÉ, 1986; CORREIA E FERREIRA 1995). Com relação à pesca, os prejuízos mais comuns são a redução das espécies de valor econômico pela predação de alevinos e danos a redes de pesca (MAEZONO E MIYASHITA, 2004).

No Brasil, *P. clarkii* é popularmente conhecido como "lagostim vermelho" ou "camarãozinho vermelho", sendo sua utilização associada principalmente ao aquarismo. Com relação a sua distribuição no Brasil, MAGALHÃES et al. (2005) e SILVA E BUENO (2005) foram os primeiros evidenciar a presença de *P. clarkii*, mais especificamente no estado de São Paulo; no qual algumas populações foram identificadas na Bacia do Rio Tietê. Com relação ao histórico e rota(s) de invasão, relatos remetem a década de 90, quando essa espécie foi bastante apreciada por aquariofilistas. No ano de 2007 o governo brasileiro decretou oficialmente a espécie *P. clarkii* como "invasora", proibindo sua introdução, importação, comercialização, cultivo e transporte (IBAMA - Portaria n°. 5 de 28 de janeiro de 2008; ver anexo 3). A legislação, todavia, permite a manutenção em domicílio de exemplares vivos como animal de estimação e em locais isolados da natureza, pelo prazo máximo de dois anos, a partir da data da publicação da Portaria. A situação exposta remete a um quadro preocupante e, possivelmente subestimado sobre ecologia de invasão da espécie no Brasil. Dessa forma, sua investigação é imprescindível para que planos de erradicação e/ou mitigação possam ser implementados.

Objetivo Geral

O objetivo desta pesquisa foi realizar uma ampla revisão bibliográfica sobre as características biológicas de *P. clarkii*, suas principais causas de introdução nos locais onde está estabelecida e averiguar sua distribuição tanto nativa quanto não nativa, a fim de compreender sua dinâmica de invasão no mundo. Adicionalmente, foi avaliada a área de ocorrência desta espécie no Brasil, especialmente no estado de São Paulo, onde já se conheciam populações estabelecidas.

Objetivos específicos

- Desenvolver uma ampla revisão bibliográfica sobre as características gerais da espécie e a problemática de invasão no mundo.
- Averiguar a distribuição da espécie no estado de São Paulo.
- Avaliar as vias de introdução da espécie no Brasil.
- Avaliar o risco de invasão da espécie no Brasil.

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–Capítulo II–

Red Swamp crayfish: Biology, ecology and invasion- a brief review

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Red Swamp crayfish: Biology, ecology and invasion- a brief review.

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Abstract.

Alien species have been transported and traded by humans for many centuries. However, with the Globalization Era, biological invasions have reached notable magnitude. Currently, alien species introduction is one of the major threats to biodiversity and ecosystem functioning. The North American crayfish *Procambarus clarkii* is one of the most widely introduced freshwater species in the world, especially due to its high economic importance. It is responsible for great modifications in invaded environments causing irreparable ecological and economic damages. Its impressive ability to successfully colonize a wide range of environments is a consequence of its behavioral and biological characteristics that can be adapted according to features of the invaded location, conferring this species a notable ecological plasticity. This review summarizes the available information regarding *P. clarkii*'s biology and invasive dynamics around the world in order to contribute to the understanding of the threats posed by its establishment, as well as support management and impact mitigation efforts.

Keywords: alien biology, exotic crayfish, invasive features, impact, invasion management.

Introduction

Human activities such as agriculture, aquaculture, recreation and transportation promote the spread of species across their natural dispersal barriers, a process which has been accelerated due to globalization. Species translocation might be intentional or accidental although most organisms die during the transport or soon after their release. Species that became invasive are those that persist, get established and cause negative effects on human health, economy, native biodiversity and ecosystem function (Kolar and Lodge, 2001).

Indeed, alien species introduction is one of the major threats to biodiversity and ecosystem functioning (Carlton, 1996; Lodge *et al.*, 2000). Some of most well known invasive freshwater alien species are the zebra mussel, *Dreissena polymorpha* Pallas, 1771, the Asian clam, *Corbicula fluminea* Müller, 1774, the Nile perch *Lates niloticus* Linnaeus, 1758 and the red swamp crayfish, *Procambarus clarkii* Girard, 1852 all of which have greatly affected rivers and lakes worldwide (Nalepa and Schloesser, 1993; D'Itri, 1997).

Procambarus clarkii, also known as red swamp crayfish or Louisiana crawfish, is native to Northeastern Mexico and the Southern U.S.A.; it has been introduced in all continents except Antarctica and Oceania, being considered the most cosmopolitan freshwater crayfish specie in the world (Hobbs, 1988; Guttiérrez-Yurrita *et al.*, 1999; Lindqvist and Huner, 1999; Gherardi, 2006; Chucholl, 2011). In a general way, this species shows good tolerance to a wide range of environmental conditions, elevated adaptive capacity, high growth rate and flexible feeding strategy (Hobbs *et al.*, 1989; Guttiérrez-Yurrita *et al.*, 1999; Alcorlo *et al.*, 2004; Souty-Grosset *et al.*, 2006; Gherardi, 2007), features that favor its establishment in new available habitats.

The present review aims to summarize and update existing information about *P. clarkii*' ecology, biology and invasive dynamics around the world. An understanding of the invasion process in different countries and habitats as well as comprehending the characteristics of the species that might favor its successful invasions can help managers to recognize the potential threats that this species pose to newly invaded ecosystems and to support management and impact mitigation efforts.

This paper is divided in nine sections that cover various aspects of *P. clarkii*'s systematics, life history, physiology, and ecology, as well as impacts observed in invaded areas. The review encompasses the traditional peer-reviewed literature, but also includes information available on reports and on the World Wide Web.

Systematics

Freshwater crayfishes are a monophyletic group of decapods crustaceans that belong to the infraorder Astacidea Latreille, 1802. They are grouped in two superfamilies: Astacoidea De Haan, 1841, which gathers the North hemisphere crayfishes, and Parastacoidea Huxley, 1879, that assembles South hemisphere species. Approximately 77% of astacidean species are distributed in North America, 20% in Australia, 1.5% in Eurasia and 1.5% in South America (Taylor, 2002; Crandall and Buhay 2008).

The superfamily Parastacoidea comprises the family Parastacidae Huxley, 1879, with representatives in South America, Madagascar and Australasia, being distinguished by the lack of the first pair of pleopods (Hobs, 1974). The superfamily Astacoidea includes the family Astacidae Latreille, 1802, with 3 genera and 12 species distributed in Eurasia and one genus in North America's West, and the family Cambaridae Hobbs, 1942, consisting in 12 genera and 337 species occurring in North and Central America and Eastern Asia (Taylor, 2002; Crandall and Buhay 2008).

Freshwater crayfishes of the genus *Procambarus* Ortmann, 1905 belong to the family Cambaridae and account for more than half of the 300 species total of cambarid crayfishes grouped in 16 subgenera (Hobbs, 1981). Most of *Procambarus* species occur in Southeastern United States and Northeasten Mexico, but there are representatives also in Belize, Guatemala and Cuba (Hobbs, 1972).

Procambarus clarkii belongs to the subgenus *Scapulicambarus* Hobbs, 1972 and even though it is native to central south USA and northeastern Mexico it has been translocated widely. In all countries, once introduced, they spread through most of the hydrographic basins (Fishar, 2006; Chucholl, 2011).

Morphological aspects

Procambarus clarkii's body, as a typical decapod crustacean, is divided in cephalothorax and abdomen, both parts having appendages following the decapod pattern (Hobbs, 1974). The abdominal appendages called pleopods aren't always present in decapod males, but in this species, they are present in both males and females (Hobbs, 1974). The thoracic appendages (pereiopods) are in number of five, as usual, but the first 3 pairs are chelate, which is a characteristic of the infraorder Astacidea (Taylor, 2002; Crandall and Buhay, 2008). The carapace color is dark red, orange or reddish brown, although blue, yellow, white and black varieties are known (Gherardi, 2011); chelae are typically red on both surfaces. Juveniles are usually light green with a narrow dark band on either side of the abdomen and a broader lighter band along the dorsal surface. Adult specimens can measure up to 15 centimeters length, although the most frequently individuals are up to 12 centimeters (Henttonen and Huner, 1999).

This specie presents external sexual dimorphism and sex can be distinguished by the position of the genital pore. The genital opening is located on the coxopodite of the third pair of pereiopods in females and on the fifth pair of pereiopods in males (Holdich and Lowery, 1988). Moreover, males have a copulatory organ formed by a modification of the first and second pair of pleopods whereas in females the first pair of abdominal appendages is vestigial and the second has no modification (Hunner, 1981).

Likewise most crayfish species, *P. clarkii* has sexual reproduction. Sexual maturity is reached in approximately three months and, depending on climate, it may produce two or three generations per year (Dorr *et al*., 2006). In adult males, two different morphotypes that alternate between each other may be observed: the reproductive form or type I male, that presents hooks on the 3rd and 4th pereiopods' ischia and more calcified copulatory organs, and the non-reproductive form or type II male, in which hooks are lacking (Taketomi *et al*., 1990; Henttonen and Huner, 1999). This morphotype alternation amid males is a characteristic of the family Cambaridae (Hobbs and Jass, 1989). An adult male might remain as type I for up to 9 months in a year. Females have no morphological alteration during reproductive phase and the only different characteristic is an increase in its receptivity to males, which might happen more than once a year depending on environmental characteristics (Sukô, 1953).

Reproduction

Mating period, as well as recruitment and sexual maturation, vary according to hydrographic period and environmental conditions (Sommer, 1984; Alcorlo *et al*., 2008) and therefore, due to the combined effects of these factors, reproduction may change after the species is introduced into different regions. Reproduction is regulated by pheromones perceived by receptors located on the antennae which are responsible for interspecific and intraspecific recognition and behavioral modulation (Ameyaw-Akunfi and Hazlet, 1975). After sex recognition, male courts female through a specific sequence of movements followed by copulation, when male turns female with dorsal part against the substrate, holding females chelipeds and both ventral regions remain in contact. Thereafter, male deposits the spermatophore in the *annulus ventralis* (not in the genital pore), which is the female's sexual receptacle, located between the bases of the posterior walking legs (Ameyaw-Akunfi, 1981).

After mating that can take days, weeks or months, also depending on environmental conditions, female safeguards herself in a burrow and starts oviposition; this process may occur in open water but is very uncommon (Holdich and Lowery, 1988). The number of eggs per brood might reach up to 300 although it depends on female size and is also related to water temperature, population density and the length of the hydroperiod (Alcorlo *et al.,* 2008). The embrionary development time depends on atmospheric temperature and may be inhibited under 10ºC (Sukô, 1953). After hatching, juveniles are kept under female's abdomen for three weeks (Holdich and Lowery, 1988).

Trophic ecology

Procambarus clarkii is a generalist omnivore species whose opportunistic diet favors its own successful establishment in different types of water bodies (Gutiérrez-Yurrita *et al*., 1999). Despite the fact that its diet in natural habitats is not widely studied, information collected on its invasive range is abundant (D'Abramo and Robinson, 1989; Feminella and Resh, 1989; Ilhéu and Bernardo, 1993; Ilhéu and Bernardo, 1995; Gutiérrez-Yurrita *et al*., 1998; Alcorlo *et al*., 2004). They are reported to feed on plant and animal detritus, macrophytes and live animals such as molluscs, insects, annelids, nematodes, platyhelminthes, tadpoles and fingerlings (Huner and Barr, 1991; Ilhéu and Bernardo, 1993; Ilhéu and Bernardo, 1995; Gutiérrez-Yurrita *et al*., 1998, Gutierrez-Yurrita *et al*., 1999; Parkyn *et al*., 2001; Correia, 2002; Buck *et al*., 2003; Cruz and Rebelo, 2005).

Regarding *P. clarkii*'s predators, the most widely cited in literature are fishes, birds and mammals like otters and capybaras (Delibes and Adrián, 1987; Holdich and Lowery, 1988); juveniles can also be eaten by odonate nymphs, coleopteran larvae and aquatic hemipterans (Gydemo and Nissling, 1990).

Causes of introduction

Shipping and aquacultural activities are the main agents of invasive alien crustacean's introduction around the world (Hänfling *et al*., 2011). Regarding alien freshwater crayfishes, shipping and ballast water are not important pathways but aquaculture and activities associated with the aquarium and bait industry are (Cohen and Carlton, 1995; Ruiz *et al*., 1997). Indeed, *P clarkii*'s aquaculture is the most important vector of introduction, being this species one of the most important freshwater decapods farmed for consumption (Huner, 1988; Hobbs and Lodge, 2010). Furthermore, this species has also been introduced as food for fishes and for other edible species like bullfrogs (Gherardi *et al*., 2011; Lodge *et al*., 2012).

In Africa, *P. clarkii* has also been introduced as a biological control agent to reduce snail's populations, which are intermediate hosts of schistosomiasis (Bilharzia) (Lodge *et al*., 2005). In addition, escapes from garden ponds and pet trade are also important introduction pathways (Dehus *et al*., 1999; Soes and Van Eekelen 2006; Dümpelmann *et al.*, 2009). Furthermore, especially in Europe, the red swamp crayfish has also been introduced to replace indigenous species, like *Austropotamobius pallipes* Lereboullet, 1858 for example, which was nearly extinct (Anastácio and Marques, 1995; Holdich, 1999).

Invasion range and history

Despite being native to Central South United States and Northeastern Mexico, *P. clarkii* has been cultured extensively through the USA and was introduced by humans in different parts of the Northern region, currently being found in Alaska, Arizona, California, Georgia, Hawaii, Idaho, Indiana, Maryland, Nevada, New Mexico, New York, North Carolina, Ohio, Oklahoma, Oregon, South Carolina and Utah (Clark and Wroten, 1978; Huner, 1986; Johnson, 1986; Ruiz *et al.*, 1997). In Northwestern Mexico, it has been successfully introduced in the states of Baja California and Sonora (Clark and Ralston, 1976; Campos and Rodríguez-Almaraz, 1992).

In Europe, *P. clarkii* was first introduced in the Iberian Peninsula, legally imported from Louisiana for commercial purposes in 1973, at two aquaculture installations in Seville and Badajoz (Habsburgo-Lorena, 1979). The commercial success led to illegal introductions throughout Portugal, Spain, France and Italy. Thereafter, it dispersed rapidly throughout the Mediterranean region and central Europe, either actively in rivers or passively by human translocations (Anastácio and Marques, 1995; Barbaresi and Gherardi, 2000). Nowadays, the red swamp crayfish is widespread and abundant in Europe, including Central and Northern European countries, where climatic features were initially considered a potential barrier to the spread of the species. Indeed, a perceptible gradient in population densities may be observed from Southwestern Europe with dense and widespread populations, to Northeastern region were populations are usually sparse and isolated from each other (Huner, 2002; Souty-Grosset *et al.*, 2006; Ilhéu *et al.*, 2007; Holdich *et al.*, 2009; Chucholl, 2011).

In Asia, this crayfish species is widely established in China, Japan and Turkey. It was initially introduced in Japan in 1927 from New Orleans, USA, as food for bullfrog aquaculture and pet trade, and now it can be found in the whole country, including Okinawa Islands (Mito and Uesugi, 2004; Kawai and Kobayashi, 2005). From Japan, *P. clarkii* was introduced to Nanjing, China in 1929 (Yan *et al.*, 2001; Li *et al.*, 2007) and has rapidly spread to most provinces of China, having established dense populations (Li *et al.*, 2005). Furthermore, a reproducing population of *P. clarkii* was also found in Israel by Wizen *et al.* (2008), but just this first record exists for this country so far.

The red swamp crayfish is also established in Africa, with records from Egypt, Kenya, South Africa, Sudan, Uganda, Zambia and Zimbabwe (Huner, 1988; Hobbs *et al.*, 1989; Arrignon *et al.*, 1990; Mikkola, 1996; Holdich, 1999; Smart *et al.*, 2002; El Zein, 2005; Foster and Harper, 2007). It was originally introduced in Kenya in 1966, from Uganda; after that, the animal's active dispersal ability and human mediated translocation resulted in its range expansion through the country (Oluoch, 1990). In Egypt, the first established population was found in the early 1980s, probably derived from a commercial aquaculture facility in Giza (El Zein, 2005; Fishar, 2006) and within the last few years, it has successfully established in various sites of the river Nile and its branches (Fishar, 2006). Regarding *P. clarkii*'s invasion in South Africa, Sudan, Uganda, Zambia and Zimbabwe, no historical information about introduction is available, just present occurrence data of established populations.

Some Central and South American countries were colonized as well, but no information about introduction pathway and date of establishment are available for most of them. In Costa Rica, invasive populations are known in the provinces of Cartago, Heredia, Alajuela, Guanacaste and Limón (Peña, 1994). *Procambarus clarkii* occurrence is also registered for Belize, Dominican Republic, Guatemala, Nicaragua, Puerto Rico and Venezuela (Huner and Avault, 1979; Huner, 1986; Hobbs *et al.*, 1989; Williams *et al.*, 2001). In Brazil, *P. clarkii* has 15 established populations, all of them in Southeast Brazil, having been introduced for aquarium trade and posteriorly released in nature accidentally or deliberately (Magalhães *et al*., 2005; Silva and Bueno, 2005).

Impacts

Procambarus clarkii occupies an important position on trophic structure of invaded environments, interacting with different trophic levels and changing the whole ecosystem functioning (Angeler *et al.*, 2001; Dorn and Wojdak, 2004; Gherardi and Acquistapace, 2007; Cruz *et al.*, 2008). Its flexible feeding strategy affects both lower and higher trophic levels by grazing on macrophytes and algae and preying on macroinvertebrates, fish fingerlings and tadpoles (Rodriguez *et al.*, 2003; Rodriguez *et al.*, 2005; Gherardi, 2006; Gherardi and Acquistapace, 2007).

Its efficiency on grazing macrophytes and extensive burrowing activity can alter freshwater environments, modifying them from macrophyte-dominated areas with clear water to phytoplankton dominated turbid areas (Rodriguez *et al.*, 2003; Geiger *et al.*, 2005; Matsuzaki *et al.*, 2009). Macrophytes are particularly important to aquatic environments because they function as service providers and ecosystem engineers (Jones *et al.*, 1994; Jones *et al.*, 1997; Duarte, 2000) avoiding erosion, facilitating nutrient cycling and providing habitat to associated faunal communities (Duarte, 2000; de Groot *et al.*, 2002; Gurnell *et al.*, 2006), and serious changes may occur in aquatic environments if submersed plant species are overgrazed. Additionally, the burrowing behavior might also cause river or channel bank erosion and increase water turbidity (Anastácio and Marques, 1997; Rodríguez *et al.*, 2003). These changes in water characteristics alter aquatic ecosystems and are believed to induce cyanobacteria blooms (Yamamoto, 2010).

Furthermore, *P. clarkii* is one of the vectors of the crayfish plague, which is mostly asymptomatic in North American crayfish species but lethal to crayfish from other regions (Souty-Grosset *et al.*, 2006; Aquiloni *et al.*, 2010; Longshaw, 2011). This disease is caused by the pathogen *Aphanomyces astaci* Schikora, 1906, a parasitic Oomycete, and constitutes a remarkable threat to indigenous crayfish species, thus being one of the leading causes of native crayfish population decline in Europe (Gutiérrez-Yurrita *et al.*, 1999; Souty-Grosset *et al.*, 2006; Holdich *et al.*, 2009).

In addition to its influence on biodiversity, *P. clarkii* can also have a considerable economic impact. Primarily, costs with ecological damages and control measures can be highlighted (Kettunen *et al.*, 2008). Costs of damage mainly occur in the agricultural, forestry, and fishery sectors. Regarding agricultural economic impacts specifically, crayfish infestation has caused serious damage to drainage systems as a consequence of its burrowing activities, causing important losses of rice yield (Sommer, 1984; Gaudé, 1986; Correia and Ferreira 1995; Anastácio *et al.*, 2000; Anastácio *et al.*, 2005).

The red swamp crayfish is also a problem for fisheries once it spoils valuable fish caught in gillnets, damages fish nets and is considered a pest in many fish ponds (de Moor, 2002; Maezono and Miyashita, 2004).

Management and control

Procambarus clarkii' invasion management options include the elimination or reduction of populations employing physical, chemical or biological methods and the use of legislation to prohibit the transport and release of specimens.

Removal campaigns using traps, fyke or seine nets and electro-fishing are commonly utilized as physical control, although they are often biased by crayfish size and sex (Westman *et al.*, 1978; Westman *et al.*, 1979). These methods are effective for population reduction but eradication is unlikely if populations aren't restricted in range and size (Gherardi *et al.*, 2011). Nevertheless, when physical control is to be used, it is better to invest in continued trapping then short-term intensive trapping, which can cause a feedback response in the population by stimulating juveniles' faster maturation and larger offspring per brood (Skurdal and Qvenild, 1986; Holdich *et al.*, 1999). Drainage of ponds is also extensively used, especially in water bodies with dense populations, as well as diversion of rivers and construction of barriers; nonetheless, the efficiency of these methods is not yet confirmed, especially for pond drainage, since *P. clarkii* is resistant to drought due to its burrowing capacity (Kerby *et al*., 2005; Gherardi *et al.*, 2011).

Another common practice to eradicate or control crayfish populations is biocide, being the application of xenobiotics, organophosphate, organochlorine, and pyrethroid insecticides the most used ones (Cecchinelli *et al.*, 2012). Chemical methods however, were found to be ineffective because of their selective efficiency, with individual crayfish being differentially affected depending on its size. In Italy, a laboratory test using the synthetic pyrethroid ciflutrin was found to be relatively effective (Quaglio *et al.*, 2002). Chemical control of crayfish activity, aiming to induce temporary inactivity, was also tested without success in rice field ecosystems (Anastácio *et al.*, 2000)

Besides being expensive, especially when applied to large areas, chemical control methods may have devastating impacts on native species and affect a wide range of organisms (Velez, 1980; Roqueplo and Hureaux, 1989). In fact, there are no selective biocides for crayfish or even crustaceans and resistance development is frequent. Furthermore, the possibility of bioaccumulation and biomagnification cannot be discarded.

Biological control methods were also employed worldwide, including the use of fish predators, disease-causing organisms and microbes that produce toxins (Holdich *et al.*, 1999; Frutiger and Müller, 2002) but the only method that has been successful so far is the use of predaceous fish like eels, burbots, perchs and pikes (Westman, 1991; Aquiloni *et al.*, 2010; Freeman *et al.*, 2010). Nevertheless, biocontrol might be risky once it may lead to new species introduction and it is not specific to the target organism, possibly also affecting native organisms as well.

All methods mentioned above present environmental costs that can overcome their benefits. In fact, any isolated method for eradication is apparently successful. Thus, a combination of methods should be considered, such as trappingand the introduction of predatory fish species.

Final remarks

Species moved beyond the limits of their normal geographic ranges by human actions usually have strong ecological impacts (Witte *et al.*, 1992; Parker, *et al.*, 1999; Hall and Mills, 2000; Latini and Petrere, 2004) and the effects of biological invasion in freshwater habitats seem to be greater than in terrestrial ecosystems, especially because freshwater invasive species have a greater tendency to disperse (Sala *et al.*, 2000; Beisel, 2001). Additionally, the importance of freshwater environments to humankind is enormous and modifications on its services will have a strong impact on human welfare.

Crayfish species have social, economic and ecological significance in several regions around the world, what favors its introduction in allochthonous areas. *Procambarus clarkii* is among these successfully and widely translocated species, and its importance is mainly associated with aquaculture and aquarium trade, being the most harvested crayfish species in the world and thus, the most intentionally introduced (Hobs and Lodge, 2010; Lodge *et al.*, 2012).

The great concern regarding this species intensive introduction is that *P. clarkii* is a successful colonizer which has specific features that increase its invasive ability and favors its colonization success across the world, in different climatic and geographic areas; these features are its ecological plasticity, adaptation of its biology and life cycle to changing environmental conditions, high tolerance to salinity, oxygen and temperature variations, high somatic growth and reproductive output, short development time and flexible feeding strategy (Alcorlo *et al.*, 2004; Gherardi, 2006; Jones *et al.*, 2009). Therefore, after established, this species may quickly become a keystone species and cause serious changes in native plant and animal communities, altering water quality and sediment characteristics (Gherardi, 2007).

In many countries, legislation designed to prevent crayfish spread is unsuccessful and conflictual due to the strong relationship between man and crayfish that results from its recreational or commercial importance. However, once introduced into favorable habitats, *P. clarkii* is rather difficult to eliminate (Holdich, 1988). Different management and control methods were cited before although their applicability and efficiency is site-specific. Unsuccessful population control is frequent and apparently solutions cannot be standardized. Being so, the most economically and environmentally effective technique is preventing introduction and range expansion.

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-Capítulo III-

Distribution, introduction pathway and invasion risk analysis of the North American crayfish *Procambarus clarkii* (Crustacea, Decapoda, Cambaridae) in Southeast Brazil

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Distribution, introduction pathway and invasion risk analysis of the North American crayfish *Procambarus clarkii* (Crustacea, Decapoda, Cambaridae) in Southeast Brazil.

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Abstract.

This study reports new records of the invasive red swamp crayfish (*Procambarus clarkii*) in Southeast Brazil and investigates its possible introduction pathways. The Brazilian crayfish pet trade was analyzed with regard to species' availability and price, reporting its persistence, even though illegal. In addition, an invasion risk analysis was performed by applying a protocol developed specifically to invasive freshwater invertebrates (FI-ISK). *Procambarus clarkii*'s confirmed occurrences are from urban and natural areas, including the Jaraguá State Park, an Atlantic Forest conservation area. Some uncertainties still remain about the extent of this species current distribution in Brazil; however, it is known that the main introduction vector is the pet trade and release of live bait by anglers may also contribute to animal's translocation. Beyond passive dispersion, these newly discovered populations, especially the ones located in natural areas, are likely to naturally disperse further through active spread and river drift of juveniles. The invasive risk analysis showed that *P. clarkii* is very likely to establish in Brazilian territory. These introductions have unknown consequences; however, many impacts are associated with this species establishment around the world, enhancing the urgency to understand this invasion process and develop efficient management techniques.

Keywords: Biological invasion, allochthonous distribution, invasive freshwater crayfish, aquarium trade, Alien species prescreening, FI-ISK.

Introduction

Alien species introduction in new habitats is considered a major threat to biodiversity and ecosystem functioning, altering autochthonous species assemblages and ecosystem processes (Stohlgren et al. 2008; Sousa et al. 2011). Freshwater environments are particularly vulnerable to invasion due to their close relation to human activities (i.e. transportation and recreation) and because freshwater species have higher intrinsic dispersal ability when compared to terrestrial organisms (Cohen and Carlton 1995; Sala et al. 2000; Beisel 2001; Vilà et al. 2010).

Native to North-eastern Mexico and South-central USA (Hobbs 1989; Henttonen and Huner 1999), the red swamp crayfish *Procambarus clarkii* Girard, 1852 has been translocated worldwide as economically important aquaculture and ornamental trade species (Gherardi 2006; Barbaresi et al. 2007; Wisén et al. 2008) and has become established, with naturalized populations, in all continents except Antarctica and Oceania (Hernández et al. 2008; Torres and Álvarez 2012).

This non-indigenous crayfish species can withstand extreme environmental conditions and is able to colonize different types of habitats (Gherardi 2007), having the ability to survive and reproduce under various biotic and abiotic conditions from tropical to temperate zones (Gherardi and Panov 2009; Siesa et al. 2011). Besides its high ecological plasticity, *P. clarkii*'s success as an invader is facilitated by its high reproductive output, short development time and a flexible feeding strategy (Gutiérrez-Yurrita and Montes 1999; Lindqvist and Huner 1999; Gherardi 2006; Alcorlo et al. 2008; Aquiloni and Gherardi 2008; Anastácio et al. 2009).

Once established in a new location *P. clarkii* can harm local benthic fauna and flora (Rodriguez et al. 2005) and cause economic losses to agricultural activities in flooded areas such as rice plantations (Correia and Ferreira 1995; Anastácio et al. 2000; Correia 2002). Documented impacts associated to its invasion include deterioration of levees and stream banks due to the burrowing behavior, decline in macrophytes, invertebrates, fishes and amphibians' biodiversity due to its feeding activities and modification of food web dynamics in invaded ecosystems (Rodríguez et al. 2005; Kerby et al. 2005; Ilhéu et al. 2007; Correia and Anastacio 2008; Cruz et al. 2008). Moreover, this species carries potential diseases to other crustaceans, such as the fungus *Saprolegnia parasitica* Coker, 1923 (Saprolegniaceae) and *Aphanomyces astaci* Schikora, 1906 (Saprolegniaceae) (Diéguez-Uribeondo and Söderhäll 1993; Gherardi and Panov 2009; Cammà et al. 2010) from which the transmission to other crustaceans species has been implicated in several indigenous species population decrease and extintions (Souty-Grosset et al. 2006; Aquiloni et al. 2011; Longshaw 2011).

The red swamp crayfish is also considered an engineering species due to its burrowing activity and intense macrophyte grazing (Angeler et al. 2001; Rodríguez et al.

2003; Anastácio et al. 2005), that alters sediment characteristics by reducing fine particulate matter, promotes bioturbation and resuspension of nutrients and sediments. In doing so it mediates matter flux and nutrient cycling, as well as impoverishes water quality and increases turbidity (Anastácio and Marques, 1997; Alcorlo et al. 2004; Creed and Reed 2004; Usio and Townsend 2004).

Many studies showed that invasion management and alien species eradication and control are tasks very difficult to achieve; additionally, environmental costs can overcome the management benefits (Hulme 2006; Gherardi et al. 2011; Januchowski-Hartley et al. 2011). Therefore, preventing introduction and range expansion is the best option to cope with biological invasion and preintroduction risk assessment has been a useful tool to forestall this threat (Lodge et al. 2012).

Dealing with invasive species requires some basal knowledge such as understanding invasion pathways of introduction and dispersion, as well as early detection and rapid assessment (Moody and Mack 1988; Rejmanek and Pitcairn 2002), so that decision makers and managers should focus their attention on newly established alien populations before they spread (Byers et al. 2002). In this perspective, it is crucial to obtain accurate assessments of location and abundance of non-native species in a way that managers can set priorities and have information to quickly and effectively combat invaders.

In Brazil, *P. clarkii* occurrence was first reported for the state of São Paulo by Huner (1986) although no specific location was provided. After that, Magalhães et al. (2005) registered five populations in São Paulo State and Silva and Bueno (2005) estimated a population of 640 individuals in Alfredo Volpi Park. This species has been sold as pet in aquarium shops since 1985 (Magalhães et al. 2005) and still has a strong trade importance nowadays despite its importation, transportation and commercialization have been forbidden since 2008 by a federal law.

Clearly, there are many gaps of knowledge regarding *P. clarkii*'s invasion dynamics in Brazil and understanding its introduction vectors and current distribution are urgent tasks. Thus, the aim of this study was to contribute to the comprehension of *P. clarkii*'s invasion process in Brazil and report the establishment of feral populations in the Southeast region, bringing new occurrence data. Additionally, the introduction pathway was also investigated and an invasion risk analysis was performed using an invasiveness screening tool.

Material and Methods

Introduction Pathway

The introduction pathway of *P. clarkii* in Brazil was evaluated based on bibliographic research through open literature and interviews with people who live or work in places where established populations are located. The total number of interviewed people was 82. Additionally, aquarium and pet shops in São Paulo state were searched online, as well as blogs from people who cultivate this species.

The range of live ornamental crayfish products of 358 online pet shops was analyzed. Shops were selected by a Google search using the Brazilian terms for crayfish, crustacean, shrimp and invertebrate. From each shop, the offered crayfish species and the corresponding price were recorded. Emphasis has been placed on the online pet trade because it is more easily accessible than conventional pet stores and because it facilitates the broad-scale spread of the offered species. The investigation was conducted over a period of 10 months, from April 2012 to February 2013.

No standardized questionnaire could be used due to the illegality of the commerce activity and therefore only qualitative data could be extracted from sellers and local people.

Allochthonous distribution

Existing occurrence data regarding *P. clarkii* distribution in Brazil were collected and this locations were visited (Table 1). From these points, field investigations were performed, what means that the locations investigated were chosen based on their proximity and connection to registered occurrence data. To verify *P. clarkii*'s presence, the margin of freshwater bodies was inspected with the help of dip nets and traps that were randomly set. The traps were baited, placed and left overnight in the water. The occurrence of burrows typically constructed by these animals was recorded as well.

Traps used in this research were already tested and considered efficient by Silva and Bueno (2005), as well as beef liver bait. All visited sites had their geographical coordinates captured with the aid of a GPS device. Three field campaigns with 10 days each were accomplished in July 2012, March and May 2013 and 35 locations were ascertained. Areas were considered colonized only when animals were captured. Evidences like people's reports and burrows' presence were considered as not confirmed indications and need further analysis (Table 2).

Table 1. Known occurrece data regarding *P. clarkii*'s distribution in Brazil, geographic coordinates (when available in the literature) and their references. (SP) - São Paulo State.

Site	Latitude (S)	Longitude (W)	Reference
Alfredo Volpi Park- São Paulo (SP)	23º35'16"	46°42'09"	(Magalães et al. 2005; Silva and Bueno 2005)
Taubaté (SP)	23°00'48"	45°33'31"	(Magalães et al. 2005)
Butantã- São Paulo (SP)	-	-	(Magalães et al. 2005)
Embu das artes (SP)	23°38'07"	46°53'45"	(Magalães et al. 2005)
Embu das artes (SP)	23°37'55"	46°53'35,4"	(Magalães et al. 2005

Invasion Risk Analysis

Invasion risk assessments are based on protocols or questionnaires that assist on predicting the possibility of some species being established and if it is likely to cause any harm. To coherently assess the invasiveness potential of *P. clarkii* in Brazil, this species was rated using the Freshwater Invertebrate Invasiveness Scoring Kit (FI-ISK, Tricarico et al. 2010). This invasion risk assessment tool helps to identify potentially invasive freshwater invertebrates by assessing their biogeography and history and quantifying the presence of "undesirable traits" according to their biology and ecology. The questionnaire comprises quantitative and qualitative elements grouped on 49 questions, which include confidence (certainty/uncertainty) rankings to each response, classifying non-native species into low-, medium-, and high-risk categories (Tricarico et al. 2010). Biogeographic, biological and ecological data used to fill in the FI-ISK form were compiled from information available in scientific journals and books obtained through an extensive literature review.

Results

According to this study, the main introduction pathways of *P. clarkii* in Brazil are the aquarium live trade and commercialization as live bait for fishing, being the latter vector more informal and less organized. Both situations are illegal and based in clandestine commerce. Thus, official information regarding live crayfish sales, as well as importation origin and rates are inaccessible.

Table 2. Sites investigated for *P. clarkii* occurrence in Southeast Brazil with coordinates (WGS84), invasion status and evidences found for its classification as colonized, not colonized or unknown. Regarding the sites, they were classified as: urban lakes (UL), fishing ponds (FP), natural areas (NA) or conservation areas (CA). Evidences used to classify the invasion status were: people's reports (R), burrows' presence (B) and captured animals (C).

Site	Latitude (S)	Longitude (W)	Status	Evidence
Santo Dias Park (UL)	23°39'53.01"	46°46'23.03"	Colonized	R, C
CEU Butantã (UL)	23°34'51.8"	46°45'03.55"	Colonized	R, C
Jardim Herculano Park (UL)	23°41'31.98"	46°45'14.22"	Unknow	B
Cordeiro Park (UL)	23°38'25.61"	46°40'39.43"	Unknow	R
Burle Marx Park (UL)	23°37'53.97"	46°43'15.18"	Not Colonized	-
Severo Gomes Park (UL)	23°38'26.9"	46°42'23.57"	Not Colonized	-
Guarapiranga Dam (UL)	26°38'32.9"	46°40'57"	Not Colonized	
Jacques Cousteau Park (UL)	23°41'60"	46°42'33.6"	Not Colonized	-
Ibirapuera Park (UL)	23°35'13.76"	46°39'44.16"	Not Colonized	-
Cidade de Toronto Park (UL)	23°30'13.4"	46°43'38.39"	Not Colonized	-
São Domingos Park (UL)	23°30'01.51"	46°44'10.74"	Not Colonized	-
Anhaguera Park (UL)	23°30'19.5"	46°43'41.1"	Not Colonized	-
Pinheirinho d´agua Park (UL)	23°26'30.31"	46°43'52.18"	Not Colonized	-
Jardim Felicidade Park (UL)	23°29'37.73"	46°43'30.03"	Not Colonized	-
Piqueri Park (UL)	23°31'42.15"	46°34'30.23"	Not Colonized	-
Chico Mende Park (UL)	23°30'25.72"	46°25'39.47"	Not Colonized	-
Carmo Park (UL)	23°34'21.68"	46°28'03.80"	Not Colonized	-
Vila dos Remédios Park (UL)	23°30'56.34"	46°44'57.46"	Colonized	R, C
Vila Guilherme Park (UL)	23°30'44.85"	46°35'54.29"	Not Colonized	-
Vila do Rodeio Park (UL)	23º34'25.83"	46º24'23.62"	Not Colonized	-
Das Águas Park (UL)	23º30'35.78"	46º22'18.58"	Not Colonized	-
Raul Seixas Park (UL)	23º33'11.47"	46º26'40.51"	Not Colonized	-
Subprefeitura de Parelheiros (UL)	23º48'23.56"	46º44'07.93"	Not Colonized	-
João Hara (FP)	23°37'13.5"	46°53'28"	Colonized	R, C
Rivieira (FP)	23°41'24.48"	46°45'04.03"	Colonized	-
Gaúcho (FP)	23°38'07.9"	46°53'45.1"	Colonized	R, C
Rancho do Netão (FP)	23º33'15.42"	47º07'32.35"	Not Colonized	-
Busca Pé II (FP)	23º49'14.84"	46º46'12.87"	Not Colonized	-
Matsumura (FP)	23º49'35.65"	46º40'19.76"	Not Colonized	-
Private area I (NA)	23°38'8.63"	46°53'43.7"	Colonized	R, B, C
Private area II (NA)	23°38'24"	46°40'39.4"	Colonized	R, B, C
APA Embu-Verde I (NA)	23º38'13.59"	46º52'29.99"	Not Colonized	-
APA Embu-Verde II (NA)	23º38'16.60"	46º52'29.72"	Colonized	R, B, C
Private area III (NA)	23º35'08.80"	47º06'22.37"	Colonized	R, B, C
Jaraguá State Park (CA)	23º27'49.47"	46º45'17.86"	Colonized	R, C

The online research revealed that *P. clarkii* is commonly sold as an ornamental aquarium species for its bright and conspicuous coloration and is also popular because of its hardiness that makes it easily cultivated and for being an aggressive animal. The red swamp crayfish is actually the only crayfish species being sold on the evaluated shops.

From the 358 aquarium shops investigated in São Paulo state, 121 sell *P. clarkii*'s specimens, which means almost 34% of the shops evaluated. Although prohibited by law, animals are readily available in online aquarium shops, and in 2012/13, the price ranged from US$2.50 for juveniles to US$6.00 for adult individuals. Additionally, none of the examined aquarium shops had information on crayfish plague or advices to prevent its posterior release in nature that would include the risks associated with the introduction of non-native species.

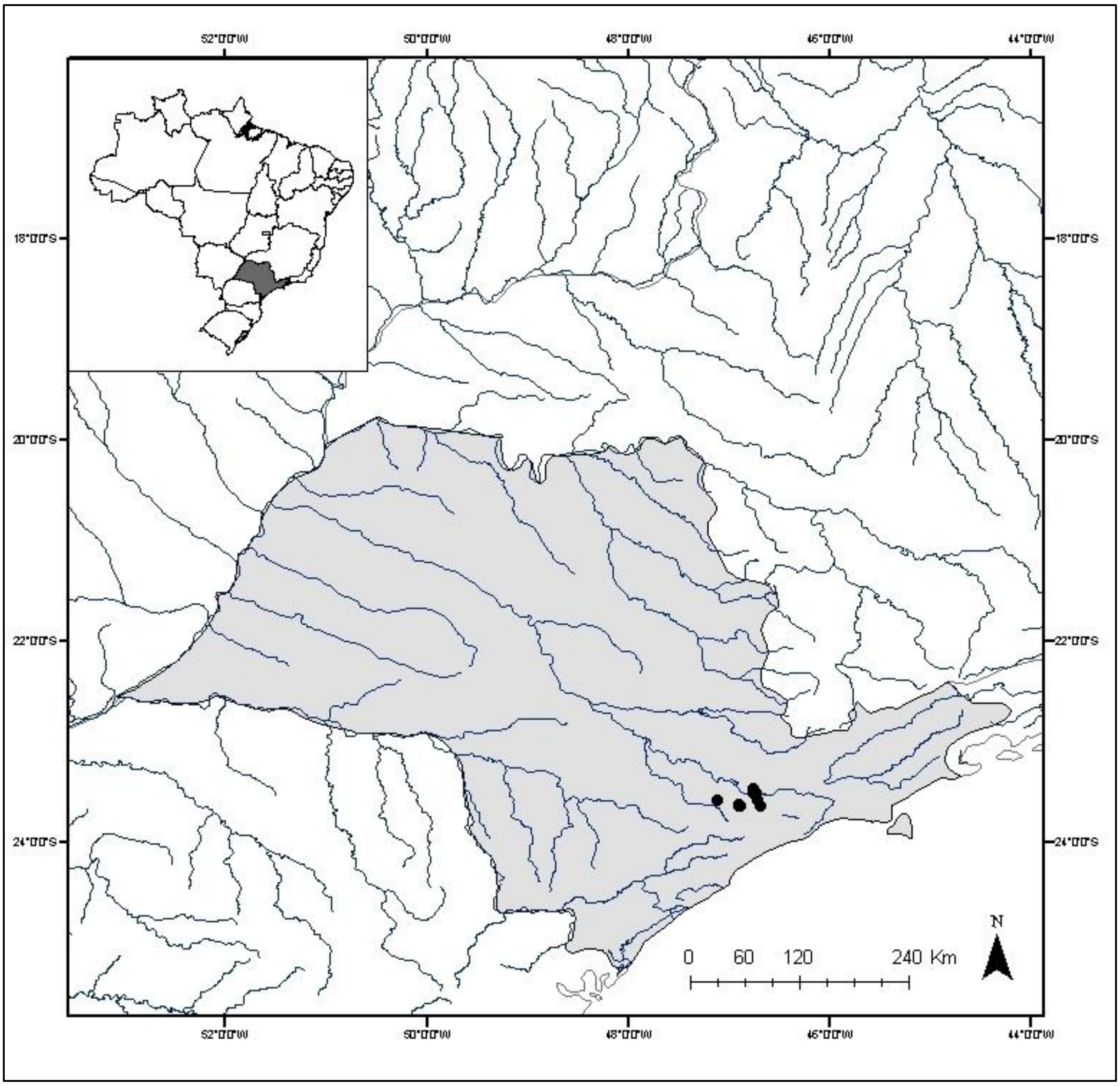

Figure 1 Map of Brazil on the upper left corner evidencing the state of São Paulo. In zoom, it is exhibited the state os São Paulo and the new *P. clarkii*'s occurrence data symbolized by dark circles. In both maps, the state of São Paulo is delineated in gray shading.

Although interviews were conducted with people related to the areas where established populations were found, it wasn't possible to determine when exactly introduction started. However, according to some sources, it is not likely to be a recent introduction, probably becoming feral sometime between 15 and 20 years ago.

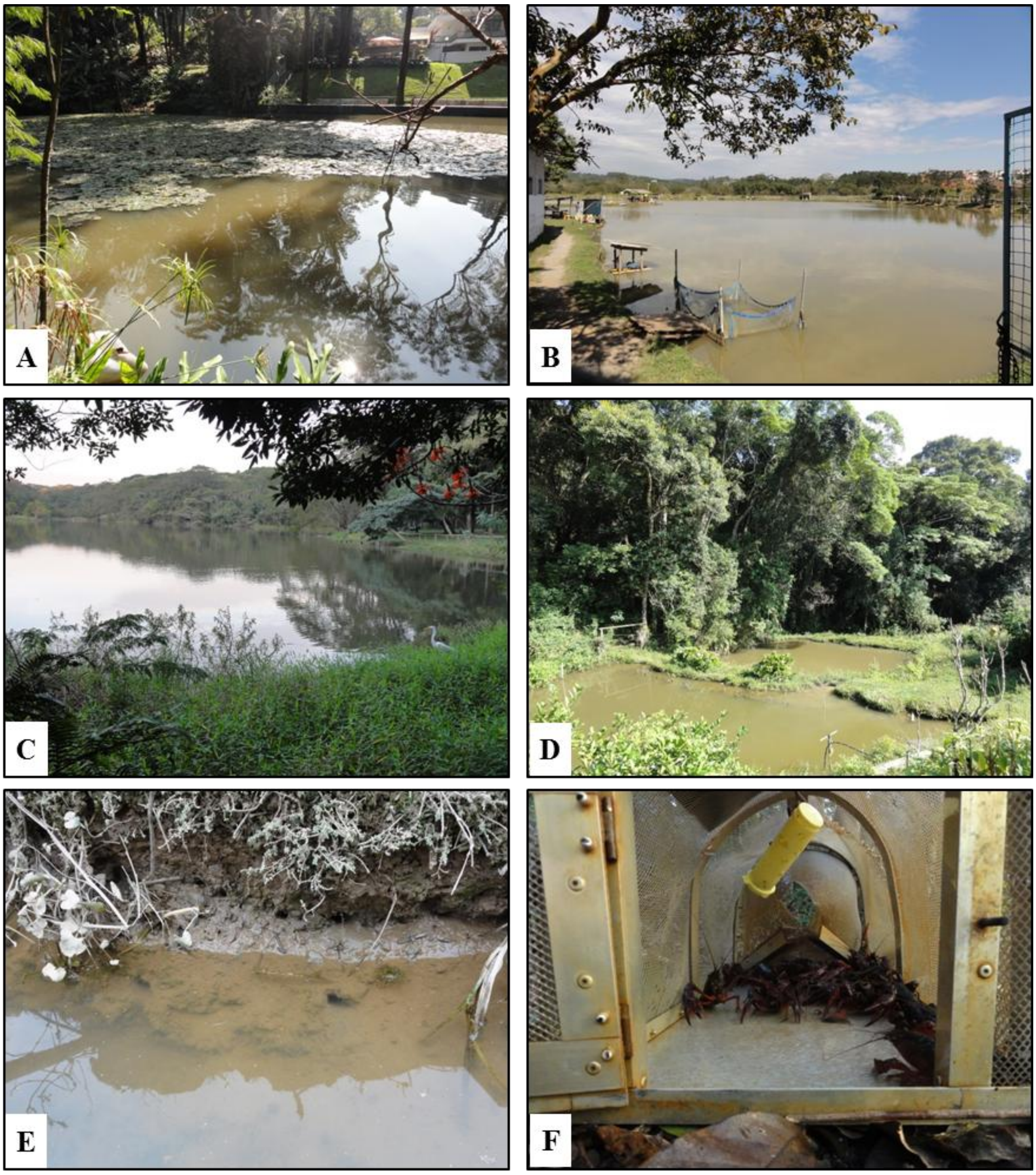

Figure 2. A-D: Sampled sites. A-urban lake (UL); B- fishing pond (FP); C- conservation area (CA); natural area (NA). E: Burrows indicating *P. clarkii*'s presence. F: Animals sampled with baited traps.

Sampling and observations of natural and artificial freshwater catchment and reservoirs in São Paulo (Table 2, Fig. 1) consistently revealed the presence of *P. clarkii* in this state. The new confirmed *P. clarkii*'s occurrences include four urban lakes, two fishponds, three natural areas and one conservation area (Table 2). Areas classified as urban lakes (UL) in table 1 are from two types: public parks located in São Paulo (capital), which have lakes or small streams, and private areas. Sites classified as natural areas (NA) are private areas, residential or used for agricultural or cattle raising purposes, located in the countryside and connected to wild areas. Fishing ponds (FP) are artificial ponds used for commercial fishing (Fig 2A-D). Each site visited was classified as colonized when animals were captured (Fig 2F); indications as burrows (Fig 2E) and reports are considered just not confirmed evidences.

Two locations were classified as unknown and further investigation is needed because in one of them the typical burrows constructed by *P. clarkii* were observed along the water body margin (Fig 2E) and, in the other, no animals were collected even though people have reported the presence of these animals (Table 1).

The invasiveness risk assessment score was 41 (Appendix 4), much higher than the trashold to consider a species as high-risk, which is 16. Thus, the FI-ISK outcome advises "reject" to *P. clarkii* introduction in Brazil.

Discussion

Understanding the factors involved in the introduction of an invasive species is of utmost importance for further evaluation of the risk posed by its establishment (Andersen et al. 2004). Unlike the main introduction pathway in the rest of the world (Lodge et al. 2000; Larson and Olden 2008), in Brazil, it seems that aquaculture and harvesting are not relevant vectors. Presently, Brazilian legislation forbids *P. clarkii* sale and transportation since 2008. Nevertheless, pet trade is the most important introduction pathway. According to Silva and Bueno (2005), *P. clarkii* specimens have been commercialized in aquarium shops in different Brazilian states for almost 20 years.

People buy this crayfish specimens to keep them as pets or for ornamental purposes in aquariums and when they give up raising them, they release the animals in freshwater environments. This is corroborated by the high prevalence of established populations in urban areas since these places are more easily accessible to release animals that people no longer want to cultivate. In addition, the invaded catchments and reservoirs containing established *P. clarkii*'s populations are widely separated from each other, being neither connected by aquatic nor terrestrial dispersal corridors. Therefore, multiple independent releases or escapes have probably occurred.

The relation between fishponds and *P. clarkii*'s invasion is still uncertain. About 50% of invaded areas are commercial fishponds but nobody interviewed knew how infestation started. Once fishes for this type of entertainment companies are purchased from big distributors, it is possible that individuals of this species are used as food for fishes and juveniles might be transported with the fingerlings that are commercialized. Once established in fishpond facylities, animals started to be used as live bait and this may have also contributed to animal's translocation.

Prevention represents the only effective method to avoid introductions of new harmful species. Invasiveness risk assessments should become an important decision tool applied by the government and decision makers when the importation or translocation of allochthonous species is to be done. The FI-ISK protocol was shown to distinguish accurately (and with statistical confidence) between potentially invasive and non-invasive species and has been widely used for crayfish species (Tricarico et al. 2009; Chucholl 2013; Papavlasopoulou et al. 2013).

The invasiveness risk assessment accomplished in this study revealed the high capability of *P. clarkii* to successfully establish and become invasive in Brazil. Therefore, precautionary techniques must be employed to avoid invaded range expansion, particularly after the study of Palaoro et al. (2013) which, using distribution models, predicted that South America territory, especially the Southeast and South Brazil, is suitable for *P. clarkii*'s establishment.

Identifying colonized areas is beneficial because the most effective time for control is when population is small (Rejmanek and Pitcairn 2002). In addition, determining the spatial extent and severity of invasions is of utmost importance (Simberloff et al. 2005). With respect to *P. clarkii*'s distribution in Brazil, there were two reports about its occurrence in the state of São Paulo in five different locations (Magalhães et al. 2005; Silva and Bueno 2005). With the new records brought by this study, the total number of occurrence sites is 15.

Procambarus clarkii seems to be locally abundant in all invaded areas. Males, females and juveniles of all sizes were captured, indicating that breeding populations are established in all visited sites. We are yet to obtain quantitative population estimates, but we believe that sufficient numbers of *P. clarkii* are present once they are frequently encountered by visitors and people that work in the areas; in addition, their burrows are abundant.

Most of the populations found in this study are established in freshwater reservoirs located in urban areas that usually have low diversity, where the impacts tend to be inferior since they already are highly modified. Nontheless, we cannot deny the importance of these areas for many birds, reptiles and amphibians. It is also very unlikely that animals will be able to naturally disperse from these urban areas once these habitats are isolated, without any connections or aquatic corridors to natural or even urban freshwater habitats.

On the other hand, in four other areas, animals were found in the wild, where reservoirs are connected by drainages to the forest streams and swamps, which provide obvious dispersal corridors. In this way, individuals belonging to these populations are likely to naturally disperse further in the region. Thus, the occurrence of *P. clarkii* in Jaraguá State Park is especially alarming because this is an Atlantic Forest conservation area that hosts many endemic and rare species (Cohen et al. 2011), including *Aegla paulensis* Schmitt, 1942. In addition, this conservation area already suffers great anthropic pressure also including fragmentation on freshwater habitats, by dam construction, pollution and deforestation among other causes.

There are no studies regarding *P. clarkii*'s impacts in Brazilian freshwater ecosystems yet. Consequently, possible impacts on native biota are only speculations for the time being, but the most probable threats are indubitably related to trophic interactions and physical disturbance according to all other invaded areas reports. One major concern is the overall reduction in the abundance of macrophytes by grazing, and for invertebrates, amphibians and fishes by direct predation on eggs, larvae, juveniles, or adults. This can result in the loss of biodiversity, which has been described widely for other invaded areas (Renai and Gherardi 2004; Cruz et al. 2005). However, it is also important to consider that several species may benefit from *P. clarkii*'s presence since this species provides an important food source for higher trophic levels such as mammals (Delibes and Adrián 1987; Matthews and Reynolds 1992; Beja 1996; Correia 2002; Tablado et al. 2010).

Numerous studies of introduced freshwater crayfish in Europe and North America showed that invasive freshwater crayfishes tend to displace native ones through mechanisms such as competition or disease transmission, leading to range contractions and occasional extinctions (Bouchard 1977; Lodge et al. 2000). Southeast Brazil has no native crayfish species, but the possible range expansion in direction to the South of Brazil where there are six endemic species of the genus *Parastacus* (Buckup 2003) is worrying.

Even though *P. clarkii* is already established in Southeast Brazil, there are opportunities to prevent this species from spreading geographically, hence preventing losses of ecosystem services. The most important thing is to invest in prevention techniques such as transmiting information and increasing public awareness. Investment in monitoring of known populations and its expansion range is also recommended.

The elaboration of management strategies is clearly needed, but a regulatory framework for the import, trade and maintenance of red swam crayfish for instance would help to alleviate the risk posed by this species' release. The availability of *P. clarkii* on aquarium shops, as demonstrated by Chucholl (2013), affects its likelihood of being introduced since it is related to a higher number of release events and thus, higher propagule pressure or introduction effort (Lockwood et al. 2007). A legislation regulating *P. clarkii*'s importation, transportation and introduction already exist in Brazil but, as we demonstrated, this species is still being sold illegally and it is also essential to invest in surveillance.

Other approaches that have been already suggested to cope with invasive crayfish and might be considered for application in Brazil are the use of tariffs to internalize invasion costs to the industries that benefit from trade of this species and the substitution of alien species by native species in aquaculture, pet industry, etc (Ewel et al. 1999).

Uncertainty remains about the extent of current distribution and we believe the invaded area is much bigger than just the sites mentioned here. Clearly, in Brazil as it is elsewhere, more extensive monitoring is required to assess newly established populations, population size and structure as well as their environmental, economical and social impacts. Additionally, investigations about local invasability are still needed in order to understand which habitat features may facilitate or avoid colonization.

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-Capítulo IV-

Considerações finais e perspectivas futuras

Considerações finais

As atividades humanas como a agricultura, aquicultura, recreação e transporte são facilitadoras para que muitas espécies possam transpor suas barreiras naturais de dispersão e especialmente após a globalização, a translocação de espécies atingiu proporções gigantescas. Dentre as espécies altamente translocadas, muitas das que são invasoras de ambientes aquáticos pertencem ao subfilo Crustacea e possuem grande potencial de estabelecimento e geração de impacto. A importância econômica deste grupo favorece a introdução intencional para aquacultura e venda de espécies ornamentais e adicionalmente, muitas espécies são transportadas desapercebidamente, com a água de lastro dos navios.

Após o estabelecimento, muitos crustáceos invasores são capazes de alterar profundamente o local colonizado e sua alta capacidade de geração de impacto se deve principalmente ao complexo papel desempenhado por estas espécies na estrutura trófica dos ambientes colonizados, onde seu estabelecimento gera efeitos em cascata capazes de alterar o fluxo de energia e nutrientes, as funções ecossistêmicas e seu funcionamento (Hänfling et al., 2011). Estas modificações afetam severamente a biodiversidade local, o ciclo hidrológico e a qualidade de água.

Trazendo á atenção a temática da invasão biológica, especialmente nos ambientes límnicos, este estudo buscou analisar o processo de invasão de *P. clarkii* no Brasil, investigando as áreas atualmente colonizadas e os vetores de introdução. De forma geral, demonstrou-se que: (1) até o momento são conhecidas 15 populações de *P. clarkii* estabelecidas em território brasilero; (2) no Sudeste Brasileiro, o principal vetor de introdução é o aquarismo e o comércio ilegal de indivíduos e (3) a análise de risco de invasão demostrou que *P. clarkii* apresenta uma alta capacidade de invasão no Brasil e sua introdução deve ser evitada, assim como prevenir a expanção das populações já estabelecidas.

Não se pode considerar que características individuais sejam preditoras da capacidade de invasão de uma determinada espécie, mas existem certos atributos que, sem dúvida, facilitam esta aptidão, são eles: a alta plasticidade ecológica a tolerância a variações de salinidade e temperatura, o hábito alimentar onívoro e o r-estrategismo. Todas estas características são encontradas em *P. clarkii*, tornando-o praticamente “o invasor perfeito”. Adicionalmente, por ser uma espécie onívora generalista, com hábito alimentar versátil, e apresentar um intenso comportamento escavador, o lagostim vermelho se torna rapidamente uma espécie chave e com capacidades de amplas modificações nos ecossistemas em que se estabelece.

O método mais efetivo e barato que pode ser utilizado para tratar do problema da invasão biológica é a prevenção, de forma que se torna imprescindível o investimento em medidas que evitem novas introduções e impeçam a disseminação da espécie. Desta forma, recomenda-se a intensificação da fiscalização sobre as lojas de aquarismo, agropecuárias e pet shops onde o comércio ainda ocorre, assim como a investigação da existência de criadouros clandestinos que abastecem este comércio. O investimento em educação ambiental é também essencial para que as pessoas que agem como atores nessa dinâmica de introdução estejam informadas sobre os danos gerados pela liberação destes animais na natureza.

Adicionalmente, os órgãos tomadores de decisão e o poder público devem favorecer a capacitação de profissionais capazes de lidar com a questão das espécies invasoras, assim como priorizar o desenvolvimento de cooperação, tanto em nível nacional quanto internacional, a fim de garantir a troca de experiências e conhecimento.

Perspectivas futuras

A confirmação referente ao estabelecimento do lagostim invasor *Procambarus clarkii* no Brasil e a descoberta de novas populações alertam para a necessidade de compreensão do processo de invasão desta espécie, os possíveis impactos gerados aos ecossistemas invadidos e a definição de táticas de manejo e controle adequadas.

Esta pesquisa foi uma investigação inicial da problemática de invasão do lagostim vermelho no Brasil e futuramente pretende-se aprofundar os seguintes temas:

- Investigação e quantificação dos impactos gerados pelo estabelecimento da espécie.

- Análise da dinâmica populacional nas comunidades invadidas.

- Verificação da capacidade de locomoção, padrões de movimento e atividade dos animais, o que permitirá a modelagem da capacidade de dispersão ativa dos mesmos.

- Análise da conectividade ambiental existente entre os ambientes invadidos e novos locais favoráveis ao estabelecimento da espécie.

- Investigação da diversidade genética das populações brasileiras e definição do histórico de invasão e populações fontes através de marcadores moleculares.

- Verificação dos limites de tolerância ecológica da espécie, por exemplo: pH, anóxia, poluição entre outros.

Anexo 1

Normas revistas Aquatic Conservation: Marine and Freshwater Ecosystems (Capítulo II).

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<u>For articles in edited volumes (e.g. books, special issues, conference proceedings):</u>

Meyer JL, Wallace JB, 2001. Lost linkages and lotic ecology: rediscovering small streams. In Ecology:*Achievement and Challenge*, Press MC, Huntly NJ, Levin S (eds). Blackwell Scientific: Oxford; 295-317.

<u>For reports:</u>

Barbour MT, Gerritsen J, Snyder BD, Stribling JB. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish. US Environmental Protection Agency Office of Water, Washington, DC.

<u>For theses:</u>

Jerling HL. 1994. Feeding ecology of mesozooplankton in the Sundays River Estuary. PhD thesis, University of Port Elizabeth, South Africa.

For European directives:

European Commission. 2000. Directive 2000/60/EC of the European Parliament and the Council of 23rd October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities* **L327**: 1-72.

For software packages:

SAS. 2002. JMP version 5 statistics and graphics guide. SAS, Cary, NC.+

For references fron the World Wide Web

Scottish Natural Heritage. 2000. http://www.snh.org.uk/ [14 June 2000]

Anexo 2

Normas revistas Biological Invasion (Capítulo III). http://www.springer.com/life+sciences/ecology/journal/10530

Author Guidelines

Biological Invasions publishes research and synthesis papers on patterns and processes of biological invasions in terrestrial, freshwater, and marine (including brackish) ecosystems. Also of interest are scholarly papers on management and policy issues as they relate to conservation programs and the global amelioration or control of invasions. The journal will consider proposals for special issues resulting from conferences or workshops on invasions.

Paper categories

ORIGINAL PAPERS

Novel empirical and theoretical research on topics in invasion biology, such as ecological consequences of invasions (including changes in community and ecosystem structure and processes), factors that influence transport, establishment, and spread of invasions, mechanisms that control the abundance and distribution of invasive species, biogeography, genetics of invaders (as genetics casts light on processes and pathways of invasions), dispersal vectors, evolutionary consequences of invasions in both historical and geological time, innovative management techniques, and analytical syntheses and overviews of invasive biotas. Authors must, in their cover letters, explain how the reported research is novel and exciting.

PERSPECTIVES, PARADIGMS, AND SYNTHESES

Overviews of policies on invasion management; perspectives on invasions and paradigms of invasion biology; syntheses of literature reports. Prospective authors should contact the Editor-in-Chief about suitability of proposed topics.

REVIEWS

Synthetic, timely reviews of topics in invasion biology for which there is a substantial literature. Prospective authors must contact the Editor-in-Chief about suitability of proposed review topic.

INVASION NOTES

Short reports (10 manuscript pages including cover page, less than 25 references, figures, and tables) of new and particularly noteworthy invasions, important changes in status or range of existing invasions, novel and promising techniques for managing particular invasions, evidence on an invasion pathway of particular interest, and the like. A note simply reporting a new species in a new location would not merit publication as an Invasion Note. Invasion Notes are not full research papers and must have (a) an abstract of one short paragraph, (b) a short introductory paragraph explaining the context of the note, (c) the reported information, and (d) a brief discussion of the significance of the note.

BOOK REVIEWS

To be solicited by the Editor-in-Chief

GENERAL INSTRUCTIONS

1) Please recall that Biological Invasions is read by specialists in invasion biology, so that introductory material pointing to the general importance of invasions is unnecessary and inappropriate.

2) Authors must submit, with their manuscripts, names and e-mail addresses of 4 unbiased, expert potential referees who have not previously read the manuscript. Authors may submit names of potential referees that they request not be used and may also request a particular handling editor.

Title Page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

- LaTeX macro package (zip, 182 kB)LaTeX macro package (zip, 182 kB)LaTeX macro package (zip, 182 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).

- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work.

- Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

- Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

- www.issn.org/2-22661-LTWA-online.php

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

- EndNote style (zip, 3 kB)

Tables

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

For the best quality final product, it is highly recommended that you submit all of your artwork – photographs, line drawings, etc. – in an electronic format. Your art will then be produced to the highest standards with the greatest accuracy to detail. The published work will directly reflect the quality of the artwork provided.

Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MS Office files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Anexo 3

PORTARIA Nº 5, DE 28 DE JANEIRO DE 2008

O PRESIDENTE DO INSTITUTO BRASILEIRO DO MEIO AMBIENTE E DOS RECURSOS NATURAIS RENOVÁVEIS - IBAMA, no uso das atribuições que lhes confere o item V do art. 22, do anexo I ao Decreto nº 6.099, de 26 de abril de 2007, que aprova a Estrutura Regimental do IBAMA, publicada no Diário Oficial da União de27 de abril de 2007;

Considerando que o *Procambarus clarkii* (lagostim-vermelho) é uma espécie exótica, capaz de colonizar ambientes diversos, com grande habilidade para dispersão e tolerante às mais adversas condições ambientais;

Considerando, ainda, que essa espécie é oficialmente reconhecida como "invasora", em mais de 30 países, e que vem sendo responsável por causar prejuízos econômicos, sociais e ambientais nos países onde se estabeleceu; e,

Considerando que a produção de *P. clarkii* no país é relativamente pequena, envolve poucos produtores e se destina, exclusivamente, ao abastecimento do mercado de Aquariofilia, resolve:

Art. 1º Não autorizar, em todo território nacional, a introdução, reintrodução, importação, comercialização, cultivo e transporte de indivíduos vivos da espécie *Procambarus clarkii*.

Art. 2º A manutenção de exemplares vivos de *Procambarus clarkii* será permitida somente em domicílios residenciais, como animal de estimação, e em locais isolados da natureza, pelo prazo máximo de dois (2) anos, a partir da data de publicação desta Portaria.

Art. 3º Fica estabelecido o prazo de sessenta (60) dias, a partir da data de publicação desta Portaria, para que criadores, empresas que comercializam animais aquáticos vivos, zoológicos e aquários públicos e particulares se adequem à presente norma.

Art. 4º Os animais não podem ser colocados em ambientes naturais, nem em águas naturais.

Parágrafo único. Os animais descartados podem ser entregues às unidades descentralizadas do Ibama.

Art. 5º Esta Portaria entra em vigor na data de sua publicação.

BAZILEU ALVES MARGARIDO NETO

Anexo 4

	Freshwater Invertebrate Invasiveness Scoring Kit (E. Tricarico, F. Gherardi & G.H. Copp)				
		Latin name:	*Procambarus clarkii*		
		Common name:	Lagostim Vermelho		
		Assessor:	Tainã		
Question ID		**Risk query:**			
		Biogeography/historical	**Reply**	**Comments & References**	**Certainty**
1	1.01	Is the species adapted for aquacultural or ornamental purposes?	Y	Geral (Huner 2002; Souty-Grosset et al. 2006; Holdich et al. 2009) - No Brasil (loureiro et al. in prep) - França (Arrignon et al., 1999; Paillisson et al., 2010; Treguier et al. 2011) - Portugal (Ramos e Pereira, 1981; Correia, 1993) - Espanha (Gutiérrez-Yurrita et al. 1999)- Bélgica (Boets et al., 2009) - Alemanha (Chucholl, 2011) - Itália (Aquilioni et al., 2011) - Egito (Fishar, 2006) - Turquia (Harliog e Harlioglu, 2006)	4
2	1.02	Has the species become naturalised where introduced?	Y	(Barbaresi et al, 2007; Wisen et al., 2008; Hernández et al., 2008; Torres e Álvarez, 2012; Henttonen e Huner,1999)	4
3	1.03	Does the species have invasive races/varieties/sub-species?	?	Sem informaçoes sobre raças, variedades ou sub-espécies.	1
4	2.01	Is species reproductive tolerance suited to climates in the risk assessment area (0-low, 1-intermed, 2-high)?	3	(Anastácio e Marques, 1995; DÖRR, 2006; Anastácio et al. 2009; GUTIÉRREZ-YURRITA e MONTES, 1999; Chucholl, 2011; Sousa et al. 2013)	4
5	2.02	What is the quality of the climate match data (0-low; 1-intermediate; 2-high)?	3	(Palaoro et al. 2013)	4
6	2.03	Does the species have broad climate suitability (environmental plasticity)?	Y	(Sommer, 1984; Gutiérrez-Yurrita e Montes, 1999; Huner, 1988; Ilheu e Bernardo, 1996; Hobbs et al., 1989; Siesa et al., 2011)	4
7	2.04	Is the species native to, or naturalised in, regions with equable climates to the risk assessment area?	Y	(Hobbs et al., 1989; Henttonen e Huner, 1999; Boets et al., 2009; Barbaresi et al, 2007; Wisen et al., 2008; Hernández et al., 2008; Torres e Álvarez, 2012)	4
8	2.05	Have introductions of the species been successful more often than unsuccessful?	Y	(Barbaresi et al, 2007; Wisen et al., 2008; Hernández et al., 2008; Torres e Álvarez, 2012; Henttonen e Huner,1999; Appleton et al., 2004; Lodge et al., 2005)	4
9	3.01	Has the species naturalised (established viable populations) beyond its native range?	Y	(Siesa et al., 2011; Schleifstein e Fedeli, 2003; Souty-Grosset et al., 2006; Gherardi e Panov, 2009; Cammà et al., 2010; Aquilioni et al., 2011; Longshaw, 2011)	4
10	3.02	In its naturalised range are there impacts to aquaculture, aquarium or ornamental species?	Y	(Schleifstein, 2003; Mueller et al, 2006; Gherardi et al, 2001; Cruz e Rebelo, 2007; Cruz et al., 2008; Gherardi e Acquistapace, 2007; Correia et al., 2002; Gherardi et al., 1999; Smart et al., 2002; Rodriguez et al., 2003; Geiger et al., 2005; Dehus et al., 1999; Gherardi, 2006; Gil-Sanchez e Alba-Tercedor, 2006; Garcia-Arberas et al., 2009; Kawai e Kobayashi, 2005; Angeler et al., 2001; Yamamoto, 2010)	4
11	3.03	In its naturalised range are there impacts to wild stocks of commercial fish and shellfish species?	Y	(Breithaupt, 1995; Mueller et al, 2006; Gherardi et al, 2001; Ilhéu et al. 2007)	3
12	3.04	In its naturalised range are there impacts to estuaries, coastal waters or amenity values?	Y	(Angeler et al., 2001; Cruz e Rebelo, 2007)	2
13	3.05	Does the specis have invasive congeners?	N	Nunca ví registro de outra espécie do gênero que seja invasora.	2

14	4.01	Does the species pose a risk to human health?	Y	Acúmulo de metais pesados (Rincon-Leon et al. 1988; Evans and Edgerton, 2002; Dickson et al. 1979; Finerty et al. 1990; King et al. 1999; MacFarlane et al. 2000; Rowe et al. 2001; Bagatto and Alikhan, 1987; Naqvi and Flagge 1990; Naqvi et al. 1990; Naqvi and Howell 1993; Reddy et al. 1994; Maranhao et al. 1995; Anderson et al. 1997; Bollinger et al. 1997; Naqvi et al. 1998; Anton et al. 2000; Anda et al. 2001)	3
15	4.02	Is it likley to out-compete with native species?	Y	(Souty-Grosset et al., 2006; Gherardi e Panov, 2009; Cammà et al., 2010; Aquilioni et al., 2011; Longshaw, 2011; Dehus et al., 1999; Gherardi, 2006; Gil-Sanchez e Alba Tercedor, 2006; Garcia-Arberas et al., 2009; Kawai e Kobayashi, 2005)	3
16	4.03	Is the species parasitic of other species or may it act a major predator on a native species that was previously subject to low predation?	Y	(Schleifstein, 2003; Mueller et al, 2006; Gherardi et al, 2001; Cruz e Rebelo, 2007; Cruz et al., 2008; Gherardi e Acquistapace, 2007; Correia et al., 2002; Gherardi et al., 1999; Smart et al., 2002; Rodriguez et al., 2003; Geiger et al., 2005)	3
17	4.04	Is the species unpalatable to predators?	N	(Adrian and Delibes 1987; Beja 1996; Correia 2001; Ruız-Olmo et al. 2002; Parkes et al. 2001; Amat and Aguilera 1988; Svardson 1972; Montes et al. 1993; Mueller and Frutiger 2001)	3
18	4.05	Is the species likely to exert a notable increased predation on any native species?	Y	(Correia, 2003; Nystrom et al., 1996; Gherardi et al., 2001; Correia, 2002; Cruz e Rebelo, 2005; Gherardi 2006; Souty-Grosset et al. 2006; Cruz et al. 2008; Ficetola et al., 2011; Irahim et al .,1995)	3
19	4.06	Does the species host, and/or is it a vector, for recognised pests and pathogens, especially non-native?	Y	Aphanomyces astaci- (Souty-Grosset et al., 2006; Gherardi e Panov 2009; Cammà et al., 2010; Aquilioni et al., 2011; Longshaw, 2011)	4
20	4.07	For crustaceans, does the species achieve an ultimately large body size (e.g > 10 cm body length) or for mussels, does the species form extensive colonies/cluster/aggregations (e.g. >1m^3)	Y	Adultos- até 15 centímetros (Dörr et al., 2006; Gherardi, 2006; Huner e Romaire, 1978; Ackefors, 1999)	3
21	4.08	Does the species tolerate a wide range of salinity regimes?	N	Toleram um pouco de salinidade, mas não muito (Gherardi e Panov, 2006; Cruz e Rebelo, 2007).	3
22	4.09	Is the species desiccation tolerant at some stage of its life cycle?	Y	(Barbaresi e Gherardi 2000; Payette e McGaw, 2003; Gherardi 2006; Gherardi et al., 2002; Cruz e Rebelo, 2007; Olden 2007)	4
23	4.10	Is the species flexible/versatile in terms of habitat use?	Y	(Hobbs et al., 1989; Siesa et al., 2011; Gherardi, 2006; Gherardi e Panov, 2006; Cruz e Rebelo, 2007; Henttonen e Huner, 1999; Anastácio et al. 2009; Oliveira e Fabião, 1998)	4
24	4.11	Does the feeding, settlement or other behaviours of the species reduce habitat quality for native species?	Y	(Correia, 2003; Nystrom et al., 1996; Gherardi et al., 2001; Correia, 2002; Cruz e Rebelo, 2005; Gherardi 2006; Souty-Grosset et al. 2006; Cruz et al. 2008; Ficetola et al., 2011; Irahim et al .,1995; Appleton et al., 2004; Siesa et al., 2011; Schleifstein e Fedeli, 2003).	4
25	4.12	Does the species require minimum population size to maintain a viable population?	?	Não há trabalho de PVA	1
26	5.01	Does the species have a wide temperature tolerance range?	Y	(Siesa et al., 2011; Gherardi e Panov, 2006; Cruz e Rebelo, 2007)	4
27	5.02	Is the species a voracious predator?	Y	(Correia, 2003; Nystrom et al., 1996; Gherardi et al., 2001; Correia, 2002; Cruz e Rebelo, 2005; Gherardi 2006; Souty-Grosset et al. 2006; Cruz et al. 2008; Ficetola et al., 2011; Irahim et al .,1995; Appleton et al., 2004)	4
28	5.03	Is the species omnivorous?	Y	(Correia, 2003; Nystrom et al., 1996; Gherardi et al., 2001; Correia, 2002; Cruz e Rebelo, 2005; Gherardi 2006; Souty-Grosset et al. 2006; Cruz et al. 2008; Ficetola et al., 2011)	4
29	5.04	Is the species planktivorous or detritivorous?	Y	(Correia, 2003; Nystrom et al., 1996; Gherardi et al., 2001; Correia, 2002; Cruz e Rebelo, 2005; Gherardi 2006; Souty-Grosset et al. 2006; Cruz et al. 2008; Ficetola et al., 2011)	4
30	6.01	Does it exhibit parental care (brooding) and/or is it known to reduce age-at-maturity in response to environment?	Y	(Paglianti e Gherardi, 2004; Lindqvist e Huner 1999; Gutiérrez-Yurrita e Montes, 1999; Gherardi, 2006; Aquiloni e Gherardi 2008; Ackefors, 1999; Dörr et al.,2006; Huner e Romaire, 1978; Jones, 1995)	4

31	6.02	Does the species produce viable gametes?	Y	(Gutiérrez-Yurrita e Montes, 1999; Gherardi, 2006; Aquiloni e Gherardi 2008)	4
32	6.03	Does the species hybridize naturally with native species?	?	Não há registros.	1
33	6.04	Is the species hermaphroditic or gynogenetic (e.g. Melanoides tubercolata or the marble crayfish)?	N	Sem registros de hermafrodiitismo. Os indivíduos que haviam sido considerados da espécie Procambarus clarkii e que são hermafrodistas vieram a ser classificados como Procambarus falax falax.	3
34	6.05	Is the species dependent on the presence of another species or specific habitat features to complete life cycle?	Y	Precisa de água.	4
35	6.06	Is the species highly fecund, iteropatric or extended spawning season?	Y	(Gutiérrez-Yurrita e Montes, 1999; Gherardi, 2006; Aquiloni e Gherardi 2008)	3
36	6.07	What is the species' known minimum generation time (in years)?	1	(Paglianti e Gherardi, 2004)	4
37	7.01	Are life stages likely to be dispersed unintentionally?	Y	(Barbaresi e Gherardi 2000; Payette e McGaw, 2003; Gherardi 2006)	3
38	7.02	Are life stages likely to be dispersed intentionally by humans (and suitable habitats abundant nearby)?	Y	Alimentação (é utilizado para alimentação em inúmeros países (Henttonen e Huner,1999), isca para pesca (Washington Department of Fish and Wildlife, 2003) e aquarismo (loureiro et al. in prep; Henttonen e Huner,1999).	3
39	7.03	Are life stages likely to be dispersed as a contaminant of commodities?	Y	Peixes- matrizes para pesqueiros.	3
40	7.04	Does natural dispersal occur as a function of dispersal of eggs and/or the movement of the suitable substratum?	?	Não achei registro bibliográfico.	1
41	7.05	Natural dispersal occurs as a function of larval or juvenile dispersal (along linear and 'stepping stone' habitats)	?	Não achei registros bibliográficos.	1
42	7.06	Are adults of the species known to migrate (reproduction, feeding, etc.)?	Y	Migração em massa de machos após o período reprodutivo (Chucholl, 2011).	4
43	7.07	Are any life stages of the species known to be dispersed by other animals (externally)?	N	Sem registros na bibliografia.	4
44	7.08	Is dispersal of the species density dependent?	?	Sem comprovação.	2
45	8.01	Is any life history stage likely to survive out of water transport?	Y	(Barbaresi e Gherardi 2000; Payette e McGaw, 2003; Gherardi 2006)	4
46	8.02	Does the species tolerate a wide range of water quality conditions, especially oxygen depletion & high temperature?	Y	(Hobbs et al., 1989; Siesa et al., 2011; Gherardi, 2006; Culley e Duobinis-Gray, 1987; Gherardi e Panov, 2006; Cruz e Rebelo, 2007)	3
47	8.03	Is the species susceptible to chemical control agents?	Y	(Gherardi & Panov, 2006; Rosenthal et al, 2005)	3
48	8.04	Does the species tolerate or benefit from environmental disturbance?	Y	(Siesa et al., 2011; Gherardi e Panov, 2006; Cruz e Rebelo, 2007)	3
49	8.05	Does the species have effective natural enemies present in the risk assessment area?	?	Sem registros nem hipóteses.	1
		Outcome:	Reject		
		Score:	41		
		Biogeography	17		
		Score partition: **Undesirable attributes**	9		
		Biology/ecology	15		
		Biogeography	10		
		Questions answered: **Undesirable attributes**	11		
		Biology/ecology	19		
		Total	40		
		Aquacultural	28		
		Sector affected: **Environmental**	31		
		Nuisance	3		
		Total questions:	49		