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**INVASÃO DE *Lithobates catesbeianus* NA MATA ATLÂNTICA SUL DO BRASIL:
RELAÇÕES COM ESPAÇO, AMBIENTE E ANFÍBIOS NATIVOS**

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1. Resumo. Invasões biológicas cresceram exponencialmente no século passado em todo o planeta, tornando-se conspícuas em quase todos os ecossistemas. Espécies invasoras podem ter pouca ou nenhuma consequência sobre as comunidades nativas em um extremo e, em outro, podem acarretar em profunda mudança estrutural, culminando com a extinção local e/ou global de muitas espécies. Entender os efeitos das invasões sobre populações e comunidades de anfíbios é uma necessidade urgente. Esse grupo tem sofrido declínios populacionais e extinções a altas taxas, e invasões contribuem para esse quadro. Anfíbios são afetados por diversos grupos de invasores, incluindo anfíbios invasores. *Lithobates catesbeianus*, a rã-touro americana, é um exemplo de espécie de anfíbio invasora. A rã-touro está presente em mais de 40 países em todo o mundo, incluindo o Brasil. A introdução da espécie está geralmente associada com as atividades de aquicultura. *Lithobates catesbeianus* é uma predadora generalista, pode ser uma competidora superior a muitas espécies nas fases larval e adulta; indivíduos podem ser vetores de diversas doenças letais para muitas espécies de anfíbios. No Brasil, a introdução da rã-touro data de 1935, quando foram importados os primeiros espécimes para a instalação do primeiro ranário do país. A ranicultura cresceu nas décadas seguintes, e os indivíduos foram transportados para diversos estados do país, para instalação de novos ranários. Aconteceram muitos escapes e liberações na natureza, que resultaram na presença de populações invasoras da espécie em vários municípios. Os objetivos da presente tese são: i) caracterizar a distribuição atual da rã-touro no país; ii) identificar as características espaciais da invasão da espécie na Mata Atlântica Sul do Brasil, quanto à abundância e presença de populações estabelecidas; iii) testar os potenciais efeitos negativos da invasão da rã-touro sobre a riqueza de anfíbios nativa; iv) testar se é possível que a rã-touro promova competição no nicho acústico com espécies nativas. Para atender tais objetivos, além de compilação de dados disponíveis na literatura (objetivo i), foram realizadas amostragens em 90 corpos d'água, em três regiões de estudo localizadas na Mata Atlântica Sul do Brasil (objetivos i, ii e iii), e foi conduzido um experimento que testou os efeitos da introdução do som do canto de anúncio da rã-touro sobre o canto de uma espécie nativa (objetivo iv). Com base nos dados coletados em campo, dados compilados de outros estudos, e registros de coleções científicas, foi possível diagnosticar que a espécie está presente em um número muito maior de localidades do que se conhecia anteriormente. Os padrões espaciais de distribuição das abundâncias de *L. catesbeianus*, em áreas da Mata Atlântica sul, são preditos principalmente por características dos corpos d'água, ou seja, fina escala espacial. Modelos espaciais e paisagem também explicam essa distribuição, mas apresentaram-se como fatores secundários. Características dos corpos d'água também foram as melhores preditoras da presença de populações estabelecidas. Os corpos d'água onde a rã-touro foi encontrada em maior abundância, não apresentaram diminuição da riqueza de anfíbios nativos. A relação entre abundância de indivíduos pós-metamórficos de rã-touro e riqueza de anfíbios nativos foi fraca e positiva. Quando foram considerados os gradientes espaciais, ambientais e de composição de comunidades, essa relação revelou-se como indireta. A rã-touro prediz a mudança do gradiente da composição que, por sua vez, prediz a riqueza. Pode-se interpretar que a rã-touro está exibindo maiores abundâncias em certas comunidades, e que essas comunidades são ricas em espécies. *Lithobates catesbeianus* mostrou-se associada a espécies comuns, de ampla distribuição geográfica, mas foi observada co-ocorrendo com diversas espécies, ainda que em menores abundâncias. Durante o experimento simulando a invasão acústica, foi observado que o canto de anúncio da rã-touro pode causar mudança no canto de anúncio das espécies nativas. Machos cantores de *Hypsiboas albomarginatus*, expostos a uma sequência de cantos de anúncio de *L. catesbeianus* com duração de cinco minutos, apresentaram alterações no tempo de emissão e na frequência de notas de seus cantos. Para muitas espécies de anfíbios anuros, o canto de anúncio é uma das bases reprodutivas, e tais alterações podem implicar em redução adaptativa. O conjunto de resultados dessa tese permitiu um melhor diagnóstico da invasão da *L. catesbeianus* no Brasil, indicando que ela encontra-se em estágios avançados de invasão. Foi possível observar que a distribuição da espécie está respondendo a filtros locais. Nenhuma relação negativa com a riqueza de espécies foi observada. Contudo, não há dados sobre essas comunidades pré-invasão para que possamos afirmar que não ocorreu perda de espécies. Os resultados do experimento de invasão acústica são um exemplo de que efeitos sobre as espécies nativas podem ser muito sutis.

2. Abstract. -Biological invasions exponentially increased in the past century in the whole world, they became conspicuous in practically every ecosystem. By one side, invasive species may have few or none effects upon native communities. In the extreme opposite, they may cause deep structural changes, resulting in local or global species extinction. Thus, it is crucial to known patterns and process related with invasions, in order to take management decisions, when necessary, and/or to prevention new invasions. The understanding of invasion effects upon amphibian populations and communities its an urgent need. This group has been suffering with high rates of population declines and extinctions, and invasions are among the causation factors of this scene. Amphibians could be affected by a variety of non-native species, including amphibians itself. *Lithobates catesbeianus*, the American bullfrog, is an example of successful invasive species. The bullfrog is present in more than 40 countries throughout the world, including Brazil. The species introduction is often associated with aquaculture activities. *Lithobates catesbeianus* is a generalist predator; it may be a superior competitor in relation with many species at larval and adult phases; individuals could be vectors of diseases, which are lethal to many other amphibian species. In Brazil, the introduction occurred at 1935, when were imported the first specimens to initiate the first bullfrog farm. The bullfrog farming expanded in following decades, and individuals were translocated to diverse new Brazilian states. Many escapes and releases occurred, resulting in the invasive populations presence in a high number of municipalities. The goals of this thesis are: i) to characterize the current bullfrog distribution in Brazil; ii) to identify the main factors prediction the invasion spatial patterns, regarding abundance and presence of stablished populations; iii) to test for potential negative effects of bullfrog invasion upon amphibian richness; iv) to test if it bullfrog calls may promote competition in the acoustic niche. To attain these goals, besides data compilation from published studies and natural history collections (goal i), samplings were carried out in 90 waterbodies, in three Southern Atlantic Forest areas (goals i, ii and iii), and it was conduced an *in situ* experiment to test the effects of bullfrog advertisement calls upon a native treefrog advertisement call (goal iv). Based on data collected in field and data compiled from other studies, it was possible to diagnose that the species is present in a many higher number of localities than previous reported. The current spatial distribution patterns of *L. catesbeianus* abundance, in Atlantic Forest Areas, are mostly predicted by local waterbody descriptors, i.e. fine spatial scale. Spatial models and landscape descriptor also predict bullfrog abundance distribution, but are secondary factors. The same waterbody characteristics were predictors of established population presence. The waterbodies where bullfrogs were found in higher abundances did not showed lower native amphibian richness. The relationship between bullfrog postmetamorphic abundances and native amphibian richness was weak and positive. When spatial, environmental and community composition gradients were taken into account, the bullfrog-richness relationship reveled to be indirect. The bullfrog predicted gradient composition change, which in its turn predicted richness. Its possible to interpret that bullfrog is showing higher abundances in certain communities, and such communities with more bullfrog individuals are species rich. *Lithobates catesbeianus* showed association with common species, with wide geographical distribution, but was observed co-occurring with a variety of species, even at small abundances. During the experiment simulating an acoustic invasion, it was observed that bullfrog advertisement call may induce change in native frogs advertisement calls. Calling males of *Hypsiboas albomarginatus*, exposed to a sequence of bullfrog advertisement calls with five min. duration, showed alterations in note duration and frequencies of their calls. Once the advertisement call is the primary reproductive selection basis for many anurans, such change may imply in adaptative loss. The set of the results presented in this thesis, allowed a better diagnostic of *Lithobates catesbeianus* invasion in Brazil, indicating that its at late invasional stages. It was possible to observe that species distribution is responding to local filter, mostly waterbody features. None negative relationship with native amphibian richness was observed. Nonetheless, there is no data about communities status before invasion to make possible to assert that species loss did not occurred in the past. The results of acoustic invasion experiment are an example that invasion effects upon native species may be subtle.

3. Apresentação

3.1. Invasões biológicas, uma breve introdução

A migração de novas espécies para comunidades locais ou para manchas de habitat inabitadas é um processo importante para o desenvolvimento de uma comunidade, bem como para a sua estrutura e composição (MacArthur & Wilson 1967; Loreau & Moquet 1999). Atualmente, nós estamos presenciando a “era das invasões biológicas”, com novas espécies alcançando comunidades via introduções intencionais ou acidentais em novos habitats promovidas pelo homem, e que, por essa razão, distinguem-se das colonizações sem interferência humana. As invasões não estão restritas a determinado táxon, região, bioma ou mesmo continente (Soulé 1990). Elas chamam a atenção por implicar em alto impacto econômico, podendo significar bilhões de dólares gastos por ano em manejo (Pimentel et al. 2000). Mas elas chamam ainda mais a atenção pelo custo ecológico, uma vez que espécies invasoras podem ser associadas à grande parte das extinções contemporâneas (Clavero & Garcia-Barthou 2005).

A era da invasão foi profetizada por Charles Elton (1958) em seu livro “*The ecology of invasions by animals and plants*” (Simberloff 2011). Influenciado pela segunda guerra mundial, Elton inicia essa obra referindo-se às invasões como “explosões biológicas”:

Ecological explosions differ from some of the rest by not making such a loud noise and taking longer to happen. That is to say, they may develop slowly and they may die slowly; but they can be very impressive in their effects, and many people have been ruined by them, or died or forced to emigrate.

Elton (1958)

Nessa obra, Elton exemplifica exaustivamente invasões nos mais diversos continentes, lança teorias sobre padrões delineando invasões e propõe muitas terminologias usadas até o presente nesse campo de pesquisa. Como disse Simberloff (2011): a elegância e o poder de persuasão de Elton garantem que o seu livro continuará sendo citado por muitas gerações de estudantes trabalhando com temas relacionados com o campo da biologia da invasão. Sintetizando e concluindo as idéias apresentadas em seu livro, Elton apresenta algumas razões para a conservação da diversidade. São elas:

- As comunidades animais com poucas espécies não atingem estabilidade populacional e estão mais sujeitas a flutuações;
- A complexidade de habitat pode alterar as dinâmicas populacionais, garantindo refúgios para populações que poderão colonizar novas áreas;
- As ilhas são mais invadidas do que as comunidades no continente;
- As invasões e rupturas populacionais ocorrem com maior frequência em áreas antropizadas, simplificadas pelo homem;
- As comunidades tropicais, ricas em espécies, estão menos sujeitas à invasão;
- O risco de invasão deve ser menor em estruturas tróficas mais complexas.

Com alguns desses argumentos, Elton inaugurou um debate acerca da relação entre diversidade e invasibilidade — propensão à invasão — dos sistemas biológicos, debate que é presente até hoje na literatura. Segundo Elton, comunidades mais ricas seriam mais resistentes à invasão porque comportariam um maior número de inimigos, predadores e parasitas, e esses fariam linha de frente perante os invasores. Entretanto, ele mesmo observou que essa era apenas uma teoria e previu que deveria ser somente parte da explicação para a aparente resistência tropical à invasão.

Com os trabalhos de MacArthur nas décadas subsequentes, a provável relação entre diversidade e invasibilidade levantada por Elton ganhou um grande reforço teórico e uma explicação mais consistente para os mecanismos de resistência das comunidades (Fridley 2011). MacArthur & Levins (1967) desenvolveram a teoria do limite da similaridade para a coexistência de espécies, que explica a diversidade local como um reflexo das diferenças entre as espécies e de como elas utilizam os recursos disponíveis. Assim, é possível prever que a invasão não será apenas barrada pelo número de espécies, mas também pelas características das espécies e a disponibilidade de recursos. Logo, é esperado que comunidades mais ricas aproveitem melhor os recursos e deixem menos espaços para espécies invasoras (MacArthur 1970).

Alguns estudos observacionais encontraram uma diminuição do número de espécies invasoras em ilhas relacionada com o aumento da riqueza de espécies, enquanto, em outros estudos, não foi observada nenhuma relação entre a diversidade e o número de espécies exóticas (Case & Bolger 1991). Dentre os resultados de estudos experimentais, são avaliáveis relações negativas (Tilman 1997), positivas (Robinson et al. 1995) e neutras (Robinson & Dickerson 1984) entre a diversidade e a invasibilidade. Após extensa revisão dos trabalhos relacionados à hipótese de Elton, Fridley et al. (2007) notaram que as relações positivas entre o número de espécies nativas e exóticas são observadas em estudos focados em grandes escalas espaciais, enquanto as relações negativas estão associadas às escalas menores, principalmente em experimentos em mesocosmos. Os autores sugerem que o paradoxo pode ser uma questão de escala espacial. Contudo, o paradoxo pode existir também em função do desenho amostral e das abordagens analíticas aplicadas nos diferentes trabalhos.

Fridley et al. (2004) demonstraram, através de um modelo nulo, que relações positivas entre espécies nativas e invasoras são esperadas simplesmente por artefatos de

amostragem. Taylor & Irwin (2004) analisaram dados de plantas invasoras e não encontraram nenhuma relação com a riqueza depois da remoção da latitude do modelo, concluindo que a relação existia em função da abordagem analítica inadequada. Assim, estudos que integrem diferentes escalas espaciais e procedimentos analíticos adequados, principalmente *in situ*, são necessários para uma melhor compreensão da relação diversidade-invasibilidade.

Elton já havia notado que distúrbios que, segundo a hipótese de Connell (1978), podem aumentar a diversidade de comunidades em frequências intermediárias também podem favorecer a invasão de comunidades (Hobbs & Huenneke 1992). Essa hipótese encaixa-se ainda na teoria de MacArthur, uma vez que distúrbios podem modificar a disponibilidade de recursos e, assim, favorecer diferentes grupos de espécies. Por exemplo, distúrbios podem alterar a qualidade dos micro-habitats, dimensão espacial do nicho, e também podem estar relacionados com a disponibilidade de propágulos de espécies invasoras (Hobbs & Huenneke 1992), sendo os propágulos mais frequentes em áreas antropizadas.

Visto que a frequência dos distúrbios pode ser considerada uma característica-chave para o processo de invasão, torna-se possível que invasões sejam mais eminentes à observação por estarem próximas a áreas com maior densidade demográfica ou, ainda, como demonstrado por Taylor & Irwin (2004), a áreas com maior produtividade econômica. Em um mundo tão modificado pelas atividades humanas, torna-se difícil separar os efeitos dos diversos fatores que contribuem para o declínio de espécies nativas, entretanto é preciso que se entenda como esses diversos fatores interagem na produção dos padrões observados na natureza (Didham et al. 2007). Ou seja, como invasão e distúrbios contribuem isoladamente e como interagem para gerar declínios populacionais.

A influência das diferentes estruturas tróficas sobre as relações que se desenvolvem entre espécies nativas e invasoras também continua sendo objeto de estudo. Estudos com espécies pertencentes a um mesmo nível trófico (e.g. Pearl et al. 2004) ou a níveis tróficos adjacentes são frequentes (e.g. Blaustein & Kiesecker 2002), entretanto a estrutura das comunidades, invadidas ou não, não é bem compreendida em estudos que modelam apenas interações entre pares de espécies. Miller et al. (2002) realizaram experimento no qual introduziram novas espécies de rotíferos e protozoários em fitotelmos, e manipularam a presença-ausência de predadores e a disponibilidade de recursos. Eles observaram que ambos os fatores, predadores e recursos, podem facilitar ou dificultar o estabelecimento de diferentes espécies.

Originalmente pensava-se que estruturas tróficas complexas poderiam barrar a invasão, mas o que acontece quando predadores de topo são os invasores? Muitos predadores são conhecidos por causar grandes impactos quando introduzidos, especialmente em ilhas. Uma das invasões biológicas mais famosas é o caso da serpente *Boiga irregularis* em Guam (Figura 1). A espécie foi introduzida na ilha durante a segunda guerra mundial. Após um período de latência, a população atingiu grandes números, e as extinções da fauna local, incluindo espécies endêmicas, iniciaram. Via predação e/ou competição, morcegos, aves, mamíferos e outros répteis foram extintos ou declinaram (Perry & Rhoda 2011).

Lagos e poças podem ser encarados como ilhas de habitat, e muitos exemplos de invasões com grandes consequências são conhecidos para esses ecossistemas aquáticos continentais (Ricciardi & MacIsaac 2011), principalmente através da introdução de peixes predadores que estão espalhados por todo o planeta (Lever 1996; Moyle & Light 1996). Um dos exemplos clássicos da invasão de peixes é a introdução de *Salmo trutta*

em riachos da Nova Zelândia. Esses peixes invasores causam o aumento da biomassa de algas, pois reduzem a abundância de invertebrados herbívoros via predação (Flecker & Townsend 1994). No Brasil, são muitos os peixes de interesse comercial que estão presentes fora dos limites espaciais das criações, e o mesmo ocorre com outras espécies introduzidas para a aquicultura, como *Lithobates catesbeianus* (Shaw, 1802), a rã-touro americana. A espécie, tema gerador desta tese, é um predador generalista e é acusada de causar declínio populacional em ambientes onde foi introduzida. Uma discussão detalhada sobre esse invasor será apresentada a seguir.



Figura 1 - *Boiga irregularis* fotografada em Western Austrália.

É importante salientar que ainda não está claro se as ilhas de ambientes aquáticos continentais são mais sensíveis aos impactos da invasão, ou se observamos um reflexo da relativa facilidade de realização de estudos nesses ecossistemas (Ricciardi & MacIsaac 2011). Também não está claro se predadores de topo são os invasores mais

problemáticos. Bartley (2011) observa que, se formos revisar com cuidado o estado trófico dos peixes invasores e os impactos associados, veremos que espécies omnívoras e herbívoras podem implicar em consequências mais adversas para os ecossistemas aquáticos do que predadores de topo. Vamos tomar o exemplo de *Cyprinus carpio*, carpa comum, espécie amplamente distribuída nos açudes e poças das zonas rurais do Brasil. Pela minha experiência de campo, consigo predizer com grande probabilidade de acerto a presença de carpa em um açude, ainda antes de confirmar a informação com o proprietário. Essa “vidência” que qualquer agricultor ou biólogo compartilha, vem da turbidez geralmente exibida nos açudes, quando a espécie está presente. A espécie, originária da Ásia e Europa, mobiliza os sedimentos, reduz a vegetação e altera a disponibilidade de hábitat para várias espécies nativas, causando uma série de consequências que podem culminar em declínio populacional (GISD 2010).

3.2. Alguns conceitos

Na busca pela compreensão de padrões e processos relacionados com as invasões, como os citados anteriormente, assim como pela urgente necessidade de manejar populações e/ou comunidades invasoras, o campo de pesquisa da biologia invasão cresceu exponencialmente nos últimos 20 anos (MacIsaac et al. 2011; Barbosa et al. *in press*). Afinal, a compreensão dos efeitos de espécies invasoras na organização das comunidades e sobre padrões e processos ecossistêmicos é fundamental para uma visão completa da paisagem no presente (Hulme et al. 2011).

Para que todos estejamos “falando a mesma língua” quando falamos em invasão, muitos trabalhos estão se dedicando à consolidação de definições, ou à construção de abordagens estruturais completas para unificar o campo de pesquisa. Eu destaco aqui a

importância de duas obras recentes: “*Encyclopedia of biological invasions*” (Simberloff & Rejmánek 2011) e “*Fifty years of invasion ecology: the legacy of Charles Elton*” (Richardson 2011). A enciclopédia da biologia da invasão pode ser tomada como um exemplo do tamanho do campo de pesquisa: são 765 páginas com conceitos, definições, exemplos, abordagens e história apresentados de forma sintética.

Já o livro editado por Richardson nasceu de um evento realizado em 2008, em comemoração dos 50 anos do livro de Elton. Esse livro tem a intenção de estimular novas pesquisas, mostrando os avanços conquistados e também o grande caminho a percorrer. No encerramento, há um capítulo dedicado à definição de conceitos e terminologias do campo da biologia da invasão. Ambas as obras constituem excelentes referências para a padronização de linguagem. A seguir, eu apresento explicitamente os conceitos de espécies nativas, introdução, invasão biológica e espécies invasoras, que foram adotados nos capítulos que seguem a essa apresentação e concordam com as definições nas obras citadas:

- *Espécie nativa* - espécie que evoluiu em uma determinada área ou alcançou a mesma sem a interferência humana (Simberloff & Rejmánek 2011);
- *Espécie invasora* - espécie que, ao alcançar uma dada região, diferente daquela nativa, lá se reproduz, frequentemente atingindo grande tamanho populacional, e se espalha de forma rápida (Richardson 2011; Simberloff & Rejmánek 2011);
- *Introdução* - liberação da espécie fora da sua distribuição nativa, seja ela intencional ou acidental (Simberloff & Rejmánek 2011);
- *Invasão* - processo multi-estágio no qual uma espécie invasora pode transpor, ou não, uma série de barreiras no contínuo naturalização-invasão (Richardson 2011).

A definição de invasão apresentada acima ainda requer maiores explicações. Pois o que é exatamente o contínuo naturalização-invasão? Quais as barreiras associadas a esse contínuo? Para explicar essas perguntas, eu vou utilizar a proposta de outra obra, também de 2011, “*A proposed unified framework for biological invasions*” de Blackburn et al. (2011). A proposta identifica algumas diferenças de abordagens dos trabalhos realizados com plantas e animais invasores e oferece uma abordagem unificada. Eu apresento uma versão em português dessa proposta na Figura 2. A partir dessa proposta, é possível observar o contínuo naturalização-invasão, ou melhor, introdução-invasão. Ela também oferece um sistema de classificação para os estágios que permite comparar as invasões e seus diversos casos, dando uma importante contribuição para que “façamos a mesma língua”. O sistema de classificação da abordagem foi traduzido na Tabela 1.

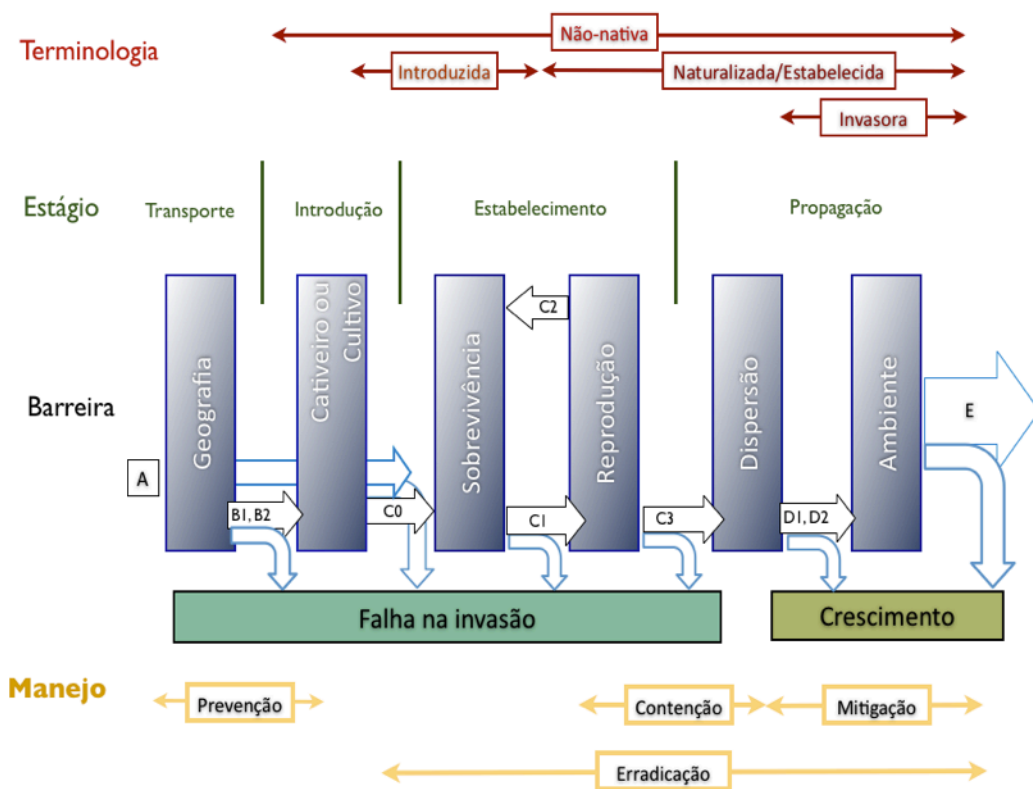


Figura 2 - Estrutura conceitual para invasões biológicas proposta por Blackburn et al. (2011). Durante a tradução eu adaptei o termo “alien” por “não-nativa”, e utilizei o termo “crescimento” ao invés de traduzir literalmente “Boom and Bust”.

Tabela 1 - Esquema de classificação para as populações na estrutura conceitual para a biologia da invasão proposta por Blackburn et al. (2011).

Categoria	Definição
A	Não transportada além dos limites da distribuição nativa.
B1	Indivíduos transportados além dos limites da distribuição nativa, e em cativeiro ou quarentena (i.e. indivíduos supridos com as condições favoráveis para eles, mas medidas explícitas para contenção).
B2	Indivíduos transportados além dos limites da distribuição nativa e utilizados em cultivo (i.e. indivíduos supridos com as condições favoráveis para eles, mas medidas explícitas para contenção são limitadas).
B3	Indivíduos transportados além dos limites da distribuição nativa, e diretamente liberados no novo ambiente.
C0	Indivíduos liberados na natureza (i.e. fora do cativeiro ou cultivo) no local onde foram introduzidos, mas incapazes de sobreviver por um período significativo.
C1	Indivíduos sobrevivendo na natureza (i.e. fora do cativeiro ou cultivo) no local onde foram introduzidos, sem reprodução.
C2	Indivíduos sobrevivendo na natureza no local onde foram introduzidos, reprodução ocorrendo, mas a população não é sustentável.
C3	Indivíduos sobrevivendo na natureza no local onde foram introduzidos, reprodução ocorrendo, população auto-sustentável.
D1	População auto-sustentável na natureza, com indivíduos sobrevivendo a uma distância significativa do ponto original de introdução.
D2	População auto-sustentável na natureza, com indivíduos sobrevivendo e reproduzindo-se a uma distância significativa do ponto original de introdução.
E	Espécie completamente invasora, com indivíduos dispersando, sobrevivendo e reproduzindo-se nos múltiplos sítios ao longo de um espectro maior ou menor de habitats e extensão de ocorrência.

3.3. Invasões biológicas: uma oportunidade...

As múltiplas invasões também podem ser encaradas como uma oportunidade científica, se considerarmos que elas constituem experimentos não planejados que

possibilitam compreender importantes processos ecológicos (Sax et al. 2007; Blackburn et al. 2011). Seria muito difícil, e eticamente questionável, que cientistas introduzissem intencionalmente novas espécies em ambientes naturais, para avaliar seu potencial de reprodução, de estabelecimento de populações e de posterior dispersão no novo ambiente (Sax et al. 2007). Todavia, uma vez que o processo de invasão está iniciado, abre-se a possibilidade de avaliar a validade de importantes hipóteses ecológicas. São justamente esses experimentos não planejados que tornam a biologia da invasão extremamente instigante, acredito eu, para muitos cientistas. Afinal, qual é a grande base de toda a biologia atual? Deixando o Theodosius Dobzhansky responder, “*nada em biologia faz sentido, exceto à luz da evolução.*”

Nós biólogos, nos mais diversos campos, estamos ancorados nas idéias de Darwin (1859) propostas em “*The origins of species*” — claro, também de Wallace e, ainda, alguns conceitos do neo-Lamarckismo. O fato é que nós estudamos células, indivíduos, populações e ecossistemas que compartilharam uma história. A realidade que observamos e tanto tentamos descrever e compreender é resultado do presente, sim, mas é também um espelho histórico: vemos o resultado da competição, seleção natural e, por que não, do acaso agindo no passado e refletidos no presente. Quando introduzimos uma nova espécie em um ambiente de cuja história ela não participou, ou ao menos seus ancestrais não participaram, abrimos uma janela de incertezas. Temos o invasor como um novo fator, que pode ser apenas mais um fator qualquer em um extremo, ou pode ser a causa da completa “quebra do espelho histórico” no extremo oposto.

O próprio Darwin considerou a questão dos invasores em novos ecossistemas e hipotetizou que invasores teriam maior facilidade de adaptação em ambientes muito distintos daqueles em que eles evoluíram, pois, nesse tipo de ambiente, constituiriam novidades evolutivas (Darwin 1859). Essa idéia é conhecida como a “Hipótese de

Naturalização de Darwin”. Quando invasões acontecem, abre-se uma janela de oportunidades para testar hipóteses como essa, as de Elton, as de MacArthur e muitas outras.

Grinnell (1919) foi um dos primeiros a reconhecer a invasão como um experimento na natureza, mas muitos outros cientistas usaram invasões para desenvolver conceitos da ecologia moderna (Sax et al. 2007). Sax et al. (2007) mostraram muitos “insights” que a invasão está fazendo emergir como “sistemas ecológicos raramente mostram evidência de estarem saturados com espécies”, “competição, diferentemente de predação, raramente causam extinção global”, ou “abordagens de envelope climático podem ser inadequadas para prever a dispersão e distribuição final de muitas espécies”, dentre outros, não menos importantes.

Nesta tese, abordando problemas relacionados a uma invasão biológica, eu vou discutir algumas das proposições de Elton (Capítulos II e III), ainda tão pouco estudadas em áreas continentais tropicais. Eu também vou discutir mais um mecanismo pelo qual a invasão pode atuar e que ainda não foi analisado: o que ocorre quando um novo som invade um ambiente? No capítulo que trata sobre esse tema (Capítulo IV), será discutida a dimensão do nicho acústico, importantíssimo para anfíbios, aves, alguns insetos e mamíferos.

Nesse grupo, a vocalização, especialmente o canto de anúncio, tem uma influência evolutiva gigantesca, pois é simplesmente a base para seleção sexual de muitas espécies (Ryan 1988). Espécies cantam em determinadas taxas, emitindo sons de determinada amplitude e frequência para atrair suas fêmeas, que podem usar essas informações para selecionar seus parceiros. Muitos estudos se dedicaram a testar a importância de competição acústica intra e interespecífica (Gerhardt & Huber 2002). E eu repito a pergunta: o que acontece quando aparece um novo som no ambiente? A ideia

da invasão acústica possibilita “invadir sem invadir”, pois podemos introduzir experimentalmente apenas o som no ambiente natural. Assim, podem ser realizados experimentos de curto e médio prazo para avaliar os efeitos da introdução nas comunidades locais, com a segurança de que não foi inserido um propágulo no meio.

3.4. *Amphibia x invasões*

A predição de vulnerabilidade de comunidades à invasão, assim como a de potenciais efeitos, ganha um caráter de urgência para a conservação de espécies da classe Amphibia, já que espécies invasoras são apontadas como uma das potenciais causas para o declínio global de anfíbios (IUCN 2011). Dentre as espécies introduzidas em ecossistemas aquáticos que afetam as populações de anfíbios, estão os peixes que podem causar grandes impactos nesses ecossistemas, conforme descrito anteriormente (Kats & Ferrer 2003). Entretanto, dentro da classe Amphibia, existem três exemplos de espécies invasoras, *Eleutherodactylus coqui* (Tomas 1966), *Lithobates catesbeianus* (Shaw 1802) e *Rhinella marina* (Linnaeus 1758), que são integrantes da lista dos “100 piores invasores do planeta”, compilada pelo grupo especialista em invasões biológicas da IUCN (GISD 2012).

Eleutherodactylus coqui é uma espécie nativa de Porto Rico, que foi introduzida na região da Flórida, nos Estados Unidos, na República Dominicana e em ilhas do pacífico (Kraus 2009; Figura 3). No Havaí, não há anfíbios e répteis nativos, teoricamente a espécie ocupou um nicho vago. As densidades médias são 20.000 indivíduos por hectare (Loope 2011). A espécie é generalista, consome uma grande quantidade de presas, e pode estar interferindo no fluxo de nutrientes dos ecossistemas (Loope 2011). Essa invasão também afeta o mercado imobiliário, pois, em função do

barulho característico da vocalização da espécie, os imóveis sofrem desvalorização com a sua presença (Beard et al. 2009). A espécie também gerou perdas para o comércio de mudas de plantas, pois as pessoas temem que, ao comprá-las, podem estar transportando indivíduos de *E. coqui* (Beard et al. 2009).



Figura 3 - *Eleutherodactylus coqui* fotografado no Hawaii. Foto gentilmente cedida por Lori Oberhofer.

Rhinella marina é uma espécie nativa das Américas Central e Sul, e foi introduzida na Austrália em 1935, para o controle de populações de besouros (Figura 4), e em pelo menos outros 40 países (Lever 2003). A espécie é generalista quanto ao uso de habitat, ocupando muitos dos ambientes não áridos disponíveis na Austrália. Essa espécie apresenta grande tamanho corporal, podendo chegar à 24 cm e pesar 2 kg (Lever 2003).

Rhinella marina expandiu muito a sua distribuição na Austrália tropical e subtropical, e a velocidade de colonização de novas áreas cresceu ao longo dos anos. Se, nas décadas de 1940 até 1960, a espécie avançava cerca de 10 km/ano pelo território australiano, as taxas de dispersão atuais são estimadas em 50 km/ano (Phillips et al. 2006b). Houve uma rápida seleção de indivíduos, e os sapos da frente de invasão apresentam pernas mais longas e se mantêm em deslocamento por períodos mais longos (Phillips et al. 2006b). Eu pude participar de um estudo conduzido por Ligia Pizzato e Richard Shine no qual era preciso considerar o movimento da espécie em Western Australia, a atual frente oeste da invasão (Figura 4), e observar indivíduos movimentando-se por mais de dois quilômetros em uma única noite. Isto é claro, assumindo-se que as movimentações ocorrem em linha reta. As predições sobre a distribuição potencial de *Rhinella marina* indicam que ela pode dobrar a sua área ocupada, deslocando-se, inclusive, através de regiões mais áridas da Austrália (Urban et al. 2007).



Figura 4 - *Rhinella marina* fotografada na frente oeste de invasão da Austrália. O cinto azul está segurando o rádio transmissor que permite rastrear a movimentação dos indivíduos. Foto gentilmente cedida por Ligia Pizzato.

Rhinella marina é a espécie de anfíbio invasora mais estudada. Porém são conhecidos os reais impactos das suas invasões? O provável impacto mais óbvio levantado historicamente foi relacionado ao consumo alimentar, que poderia ser maior ou diferente daquele dos anuros locais (Greenless et al. 2006). Entretanto, mesmo uma questão muito básica como essa permaneceu, até pouco tempo atrás, sem um teste sistemático. Greenless et al. (2006) dedicaram-se a responder essa questão e verificaram que os caminhos dos impactos da invasão poderiam ser bem distintos daqueles esperados.

Os resultados de Greenless et al. (2006) indicaram que os sapos invasores realmente reduziam a biomassa e riqueza das presas, no caso, os invertebrados terrestres, mas o faziam em taxas similares àquelas das espécies nativas. O experimento apontou, ainda, que, com a chegada dos invasores, a biomassa da fauna nativa permanecia a mesma. Como consequência da presença do invasor, ocorreu apenas o acréscimo na biomassa total de anura no ecossistema, e não redução como poderia ser esperado. Tendo em vista que *R. marina* é uma espécie tóxica para os predadores locais (Phillips et al. 2006a), os autores concluíram que a espécie poderia ser considerada um “sumidouro” de biomassa (Greenlees et al. 2006).

As principais toxinas de *Rhinella marina* são os bufadienolides, compostos cardiotoxicos exclusivos dos sapos (Chen & Kovarikova 1967). Na Austrália, esses compostos não são encontrados nas espécies nativas, e, conseqüentemente, a chegada de *R. marina* no continente possibilitou o teste da adaptação de predadores a uma nova presa potencial com diferentes tipos de defesas (Phillips & Shine 2006). Muitas espécies de serpentes, importantes predadores de anfíbios, sofreram o impacto da invasão de *R. marina*, visto que, frequentemente, morreram após o consumo de indivíduos dessa

espécie (Phillips & Shine 2006). Contudo, a adaptação à presença do invasor foi muito mais rápida do que o esperado. Quando indivíduos de *R. marina* foram oferecidos às serpentes coletadas em áreas invadidas e não invadidas em um experimento, nenhuma serpente que já convivia com a espécie na natureza consumiu o sapo, enquanto metade das serpentes que não conviviam com a espécie no ambiente o consumiram (Phillips & Shine 2006).

Estudos sobre competição, nos quais os potenciais impactos de *Rhinella marina* sobre outras espécies de anuros foram quantificados, foram realizados recentemente. O estudo de Greenlees et al. (2006) não mostrou alteração na biomassa de anuros nativos na presença de *R. marina*. Tendo em vista esse resultado, Greenlees et al. (2007) testaram se havia alteração no forrageamento de uma espécie nativa e se, ocorrendo tal alteração, esta seria causada por exploração de recursos ou por interferência. Os resultados mostraram que a presença do invasor não alterou o consumo de presas, entretanto o invasor reduzia a atividade da espécie. Nesse estudo, novamente, os efeitos indiretos causados pelo invasor foram sobressalientes em comparação com aqueles esperados pelo senso comum.

Lithobates catesbeianus, a rã-touro, é outro exemplo de invasor de sucesso da classe Amphibia. A espécie é nativa do leste da América do Norte (Canadá e Estados Unidos), mas foi introduzida em diversos países para fins de consumo humano (Figura 5). *Lithobates catesbeianus* exibe traços que favorecem a invasão: tamanho corporal grande, dieta ampla, capacidade de atingir alta densidade populacional, alta fecundidade e rápida maturação sexual (Pearl et al. 2004; Kaefer et al. 2007). Há trabalhos que apontam uma habilidade competitiva superior dos girinos de rã-touro em relação às espécies nativas, assim como um forte impacto de predação dos adultos sobre outras

espécies (Kiesecker & Blaustein 1998). Portanto, os potenciais efeitos da interação da rã-touro com a anurofauna de uma comunidade invadida, aqui, já se distinguem daqueles relatados para *Rhinella marina*.

A rã-touro consome itens alimentares componentes da dieta de outras espécies, configurando-se uma potencial competidora, mas também é potencial predadora dos seus competidores. Entretanto, para a rã-touro, assim como para outros invasores, há pouca evidência de que a predação afete populações, embora haja muitos estudos observacionais que indicam que a espécie pode afetar abundância de populações inteiras (Kraus 2009). Além da competição e da predação, há relatos de efeitos indiretos da rã-touro sobre espécies nativas, como a indução de alteração no uso de microhabitats de outras espécies, o que torna as espécies nativas mais expostas à predação (Kiesecker & Blaustein 1998; Pearl et al. 2004).



Figura 5 - Bruno Madalozzo segurando um indivíduo de rã-touro, *Lithobates catesbeianus*, coletado na borda do Parque Nacional da Serra do Itajaí, em Santa Catarina, no ano de 2010.

Uma comunidade invadida pela rã-touro pode ser invadida, imediatamente, por toda uma comunidade microbiótica indesejável. A espécie pode carregar muitos parasitas (Bury & Whelan 1984) e tem sido apontada como possível vetor do fungo *Batrachochytridium dendrobatides* (Daszak et al. 2004), o agente causador da quitridiomíose, associado ao declínio e extinção de anfíbios em todo mundo (Schloegel et al. 2010). Experimentalmente, foi demonstrado que indivíduos de *Lithobates catesbeianus* com a infecção por *B. dendrobatides* confirmada apresentam lesões pequenas e concentradas, com taxa de letalidade associada insignificante (Daszak et al., 2004). Garner et al. (2006) tomaram amostras de diversas populações da espécie, inclusive do Brasil, principalmente populações invasoras, e observaram que as amostras portavam *B. dendrobatides*. Já Toledo et al. (2006) coletaram amostras de uma população invasora e de uma criação de rãs, nas quais não detectaram a presença de *B. dendrobatides*.



Figura 6 - Mapa indicando os municípios onde há registro de populações invasoras da rã-touro no Brasil. Mapa produzido no Google Mapas, disponível em http://www.ib.usp.br/grant/Ra-touro_no_Brasil/Pagina_Inicial.html.

Lithobates catesbeianus está presente no Brasil e em diversas localidades (Figura 6) e é a invasora protagonista desse trabalho. A seguir, são detalhados aspectos da história de vida da espécie e o estado de conhecimento da sua invasão no país.

3.4.1 Um pouco sobre a história de vida de *Lithobates catesbeianus*

Indivíduos de *Lithobates catesbeianus* apresentam grande tamanho nas fases larval e adulta. Os adultos apresentam dimorfismo sexual, sendo que o diâmetro do tímpano dos machos é maior que o diâmetro do olho e apresenta coloração marrom escura nas bordas, enquanto as fêmeas apresentam os diâmetros do tímpano e do olho aproximadamente do mesmo tamanho (Bury & Whelan 1984; Figura 7). As fêmeas chegam a 200 mm e são maiores que os machos, que alcançam até 180 mm (Bury & Whelan 1984).

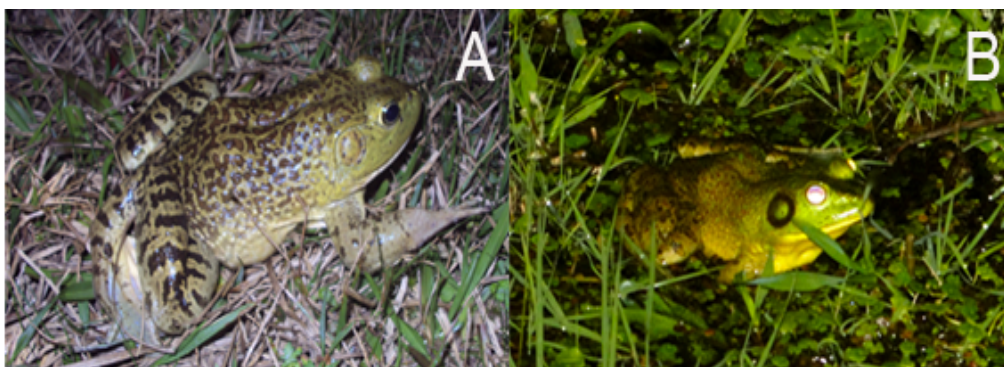


Figura 7 - Fêmea (A) e macho (B) da rã-touro. Note o papo amarelo do macho e o tímpano escurecido. A foto A foi uma cortesia de Camila Medeiros.

Pode-se dizer que a dieta larval ainda não é bem conhecida. Relatos antigos indicam que os girinos de rã-touro alimentam-se de plantas aquáticas, alguns invertebrados, algas, peixes e girinos em decomposição, ovos de anuros e fezes de larvas

da própria espécie (referências em Bury & Whelan 1984). Estudos recentes parecem contrariar o mito da herbivoria, hábito alimentar mais comumente atribuído a girinos, e chamam a atenção para a variabilidade espaço-temporal da dieta (Altig et al.2007). Já a dieta dos adultos recebeu uma grande atenção no mundo acadêmico e rendeu à espécie a fama de voraz. A espécie é generalista, predando uma grande quantidade de presas, desde macroinvertebrados até vertebrados de médio porte. Dentre as presas conhecidas: sanguessugas, insetos e outros artrópodes, peixes, lesmas, salamandras, girinos, pequenos crocodilos, pequenas tartarugas, serpentes, morcegos, ratos e pássaros (Boelter & Cechin 2007; outras referências em Kraus 2009).

A reprodução da espécie é descontínua em grande parte da sua distribuição nativa, entretanto é potencialmente contínua em regiões subtropicais (Kaefer et al. 2007). A vocalização dos machos nas áreas nativas parece iniciar com o aumento da temperatura e aquecimento da água, quando a última atinge temperaturas iguais ou superiores a 14°C (Bury & Whelan 1984), na distribuição norte e sudeste do Canadá e nordeste dos Estados Unidos, enquanto nas áreas mais ao sul e sudeste dos Estados Unidos e México, a espécie pode estar ativa ao longo de todo o ano. O canto de anúncio dos machos cobre uma ampla faixa de frequências, de 90 até 4000 Hz. Há dois picos de concentração de energia, o primeiro entre 200 e 400 Hz, e o segundo entre 1000 e 2000 Hz (Capranica 1968).

Os machos da espécie podem exibir três estratégias reprodutivas: territorialistas, oportunistas e “satélites” (Bury & Whelan 1984). Os territorialistas são os machos maiores que cantam e defendem ativamente o território. O oportunismo é utilizado por machos maiores de idades intermediárias, que cantam sem defender os territórios. Já os denominados machos satélites são machos jovens e pequenos que não cantam tampouco

defendem o território, mas ficam próximos de machos cantores para interceptar fêmeas eventualmente. Estudos apontam a existência do reconhecimento intraespecífico individual através dos cantos (Bee & Gerhardt 2002). Também é reportado que as agressões entre machos, conhecidas para a espécie, podem ser mais intensas na presença de indivíduos estranhos (Bee & Gerhardt 2002). Os amplexos ocorrem na água, e os ovos são depositados em um filme gelatinoso na superfície (Figura 8A). As desovas podem conter entre 1000 e 25000 ovos (Bury & Whelan 1984). Os girinos podem passar dos 15 cm de comprimento, apresentam coloração verde-oliva, com pontos escuros no dorso (Figura 8).



Figura 8 - Desova da rã-touro em A, girinos em diferentes tamanhos e estágios de desenvolvimento em B, C e D. Fotos disponíveis no site Rã-touro no Brasil.

3.4.2. *O que sabemos sobre a invasão da rã-touro no Brasil?*

Antes de ser introduzida no Brasil, a rã-touro já era explorada comercialmente em Cuba, onde foi introduzida em 1917 e espécimes foram liberados na natureza onde eram caçados (Lima & Agostinho 1988). Uma década depois, o Japão introduziu a rã-touro, quando o próprio governo distribuiu lotes para rizicultores (Lima & Agostinho 1988). Na década de 1930, foi a vez de o Brasil iniciar a prática da ranicultura. A introdução data de 1935, quando foram importados cerca de 300 casais dos Estados Unidos para a instalação de um ranário no estado do Rio de Janeiro (Lima & Agostinho 1988). Diferentemente de Cuba e Japão, aqui a criação foi voltada para o cativo. Lima & Agostinho (1988) descrevem assim o início da criação de rãs no país:

“O primeiro registro de importação da rã-touro data de 1935, e nove anos depois surgiu o Ranário Aurora, com tanques de formas variadas, semelhantes aos utilizados na criação de peixes, com certa área de terra cercada por folhas de zinco ao seu redor”.

Lima & Agostinho (1988)

A atividade permaneceu restrita no país até a década de 1970, quando gerou grande interesse dos criadores, despertado por reportagens de jornais e revistas relatando o sucesso dos ranicultores e prevendo grande faturamento (Lima & Agostinho 1992). Na década seguinte, já havia cerca de 2000 ranários no país, e entre 1978 e 1986, ocorreram cinco Encontros Nacionais de ranicultores, onde se encontravam os criadores pertencentes às recém formadas cooperativas e associações (Lima & Agostinho 1988). Porém a ranicultura começou rudimentar no país, já que muitos dos primeiros criadores

não tiveram as orientações adequadas para o manejo e foram desistindo da atividade em razão do baixo lucro (Lima & Agostinho 1992). O cenário vem se modificando desde o final da década de 80. As cooperativas, o avanço científico-tecnológico e a “fome de rãs” vêm consolidando o setor como uma atividade importante (Lima & Agostinho 1988). Na Figura 9, são apresentadas algumas fotografias de sistemas de ranários presentes nos municípios de Ponte Nova e Viçosa, em Minas Gerais.



Figura 9 - Fotos de ranário desativado em Ponte Nova, Minas Gerais, em A e B. As demais fotos são do ranário experimental da Universidade Federal de Viçosa, mostrando tanque com girinos (C), indivíduo metamorfo (D), e indivíduos atingindo tamanho/peso para o abate em E e F. Todas as fotos são de Emanuel Teixeira da Silva.

Grande parte da produção nacional é destinada à exportação, e, dentro do Brasil, o consumo não é muito frequente, mas ocorre. O custo de 500g de coxas de rã-touro congeladas é de aproximadamente R\$ 30,00. Em um bar à beira-mar, em Santa Catarina, o petisco sai por aproximadamente R\$ 35,00 (Figura 10).



Figura 10 - Fotografia de cardápio oferecendo carne de rã-touro como petisco à beira-mar em Santa Catarina.

“Existem dois tipos de consumidor de carne de rã no Brasil _ a população de baixa renda, que obtém o produto por meio da caça (embora seja proibida por lei), e as classes mais favorecidas, que se abastecem nos supermercados e restaurantes.”

Lima & Agostinho (1988)

Podemos imaginar que a invasão das rã-touro no Brasil tenha iniciado a ganhar forma na década de 70 — pois quem garantia que elas não escapariam dos cativeiros? — e que tenha crescido significativamente nas décadas subsequentes, à medida que as criações foram sendo abandonadas (Giovanelli et al. 2008). Falando com proprietários rurais do Rio Grande do Sul e Santa Catarina, eu ainda identifiquei um caminho diferente para as introduções recentes, também relacionado à aquicultura. Os proprietários, às vezes, ganham girinos da rã-touro quando vão comprar alevinos para abastecer os seus açudes. Eles dificilmente conhecem a história da rã-touro, sequer as potenciais consequências da sua introdução nos açudes. As rãs podem predação muitos alevinos, e talvez por isso virem “presentes”. Nesses últimos quatro anos, coletando e ouvindo histórias, o colega Rodrigo Lingnau se deparou com a seguinte situação: um guarda-parque, vendo girinos agonizando em um açude que estava secando, resolveu salvar os girinos e colocou-os em um balde para liberá-los em um riacho. Com base nessas informações, podemos dizer que hoje a falta de informação continua favorecendo a invasão da rã-touro no Brasil.

No estado do Rio Grande do Sul, o primeiro registro de *Lithobates catesbeianus* na natureza publicado foi para o Parque Estadual do Turvo em Kwet (1999, apud. in Borges-Martins et al. 2002). Borges-Martins et al. (2002) publicaram uma série de novos registros para o estado, incluindo o primeiro registro para Santa Catarina. Giovanelli et al. (2008) ampliaram mais ainda o registro da distribuição atual das populações invasoras no Rio Grande do Sul e no Brasil inteiro, o que forneceu uma noção melhor do tamanho da invasão. Entretanto, há muitas lacunas de informação acerca dos padrões temporais da invasão. Visto que a invasão se dá por múltiplas e sucessivas introduções, de muitos ou poucos indivíduos, e também há algum grau de dispersão, há uma grande dificuldade para caracterizar a história da invasão da espécie.

Nos últimos 10 anos, foram publicados diversos estudos citando a rã-touro, incluindo muitos levantamentos de fauna (lista completa no capítulo I). Dentre estudos de história natural das populações invasoras, destacam-se o trabalho de Kaefer et al. (2007), que descreve o ciclo reprodutivo da rã-touro na natureza e identificando o potencial para a distribuição contínua em áreas subtropicais, e os trabalhos de Boelter & Cechin (2007) e Silva et al. (2009), que descreveram os itens alimentares da dieta da rã-touro e observaram que anfíbios anuros e invertebrados aquáticos são frequentes na dieta da rã. Silva et al. (2011) analisaram ainda a relação entre presas de anura encontradas na dieta de rã-touro e a similaridade de microhabitat entre a rã e respectivas presas. Esse estudo identificou que as espécies predadas apresentam alguma similaridade de uso de microhabitat com a rã, entre média e alta, e observou que a predação pode ser maior ainda em áreas preservadas.

Quatro trabalhos avaliaram a distribuição potencial da rã-touro no país e/ou na Mata Atlântica. O primeiro estudo foi de Ficetola et al. (2007), que utilizou descritores climáticos atuais e “pegada humana” como índice de antropização para modelar a distribuição da espécie em todo o planeta. Os resultados indicaram que as regiões sul e sudeste do Brasil são especialmente favoráveis para o estabelecimento da espécie. Giovanelli et al. (2008) modelaram a distribuição potencial no Brasil usando descritores climáticos e topográficos e encontraram resultados bastante similares aos de Ficetola et al. (2007). Os autores ressaltaram a importância de estudos sobre a invasão da rã-touro na Mata Atlântica, que concentra grande diversidade mundial de anfíbios. Nori et al. (2011), além de modelarem a distribuição da espécie de acordo com o clima atual, modelaram a potencial distribuição da espécie em 2050 e 2080, de acordo com diferentes previsões de mudanças climáticas. Esse estudo reforçou os resultados anteriores e apontou que, mesmo com as mudanças climáticas, a Mata Atlântica,

especialmente no sul do país, permanecerá favorável para populações invasoras da rã-touro. Nori et al. (2011) e Loyola et al. (2012) compararam a distribuição potencial da espécie com o mapa de áreas protegidas na América Latina e Mata Atlântica, respectivamente, e demonstram que, no futuro, as áreas protegidas do bioma podem ser ainda mais favoráveis para o estabelecimento de populações da rã.

Há diversos relatos de observações pontuais ou estudos descrevendo a invasão em locais específicos, mas a minha intenção nesta apresentação não é fornecer uma lista completa de casos. A literatura sobre ranicultura também é imensa, basta observar a página eletrônica do grupo de pesquisa da Universidade Federal de Viçosa, referência no país, ou da própria Embrapa, para dimensionar o tamanho desse campo de pesquisa e o potencial do setor econômico. Novamente, descrever a ranicultura no Brasil também não cabe aqui. Resumindo, com essa breve apresentação, eu quero apenas chamar atenção para o fato de que pesquisadores da área da aquicultura vêm estudando técnicas de criação da rã-touro há mais de 30 anos, e nós estamos no mínimo 20 anos, eu diria 30 anos, atrasados quanto ao conhecimento das populações invasoras. O nosso conhecimento sobre essa invasão é tão pequeno, que, até o momento, somente conhecemos alguns locais onde há a presença de populações invasoras, alguns aspectos da dieta da espécie no país e um pouco sobre a biologia reprodutiva em áreas invadidas. Sabemos, também, que a espécie encontra aqui condições climáticas favoráveis e que áreas preservadas podem ser ainda mais favoráveis para a espécie no futuro.

3.5. Objetivos da tese

Os estudos abordando história natural da rã-touro, ou melhor, sua história natural em áreas invadidas, e padrões da invasão vêm crescendo no Brasil e na América Latina,

nos últimos anos, acompanhando a tendência mundial de crescimento de estudos sobre biologia da invasão (MacIsaac et al. 2011). Aqui no Brasil, porém, estudos que avaliem os padrões espaciais da invasão *in situ*, ou que quantifiquem um provável impacto da espécie sobre as comunidades locais, ainda não foram realizados. Tampouco estudos testando os potenciais efeitos da invasão sobre espécies nativas em condições experimentais. Tais estudos são fundamentais para o planejamento de medidas de conservação, tão aclamadas por colegas citados acima, se estas forem cabíveis, e constituem um passo essencial para o delineamento de futuros projetos que abordem questões mais específicas relacionadas com o processo de invasão da espécie.

Considerando o que sabemos e a grande necessidade de se conhecer os padrões e processos operantes na invasão da rã-touro, a presente tese tem os seguintes objetivos:

I. Caracterizar a distribuição atual da rã-touro no país.

II. Identificar as características espaciais da invasão da espécie na Mata Atlântica sul do Brasil, avaliando a importância de descritores espaciais e ambientais na predição da abundância da espécie e na presença de populações estabelecidas.

III. Testar os potenciais efeitos negativos da invasão da rã-touro sobre a riqueza de anfíbios nativa, considerando potenciais descritores espaciais e ambientais que podem estruturar essa relação.

IV. Testar se é possível que a rã-touro promova competição no nicho acústico com espécies nativas, avaliando a influência do canto de anúncio da rã-touro sobre uma espécie nativa.

Cada um dos objetivos supracitados é atendido em um capítulo nesta tese. Os capítulos são:

Capítulo I. “Widespread occurrence of the american bullfrog, *Lithobates catesbeianus* (Shaw, 1802) (Anura: Ranidae), in Brazil.”

Artigo publicado no South American Journal of Herpetology, 2011, 6:127-134.

Capítulo II. “Fine-scale environmental descriptors predict invasive *Lithobates catesbeianus* distribution in the Brazilian Atlantic Forest.”

Artigo submetido para o Journal of Applied Ecology.

Capítulo III. “Amphibian richness in Atlantic Forest invaded areas by american bullfrogs: may we expect low native richness where bullfrogs are abundant?”

Artigo com submissão prevista para a Biological Invasions.

Capítulo IV. “Biological invasions and the acoustic niche: the effect of bullfrog calls on the acoustic signals of white-banded tree frogs.”

Artigo aceito para publicação na Biology Letters, publicação on-line prevista para 06.06.2012.

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4. Capítulo I

**Widespread occurrence of the American bullfrog, *Lithobates catesbeianus*
(Shaw, 1802) (Anura: Ranidae), in Brazil**

WIDESPREAD OCCURRENCE OF THE AMERICAN BULLFROG, *LITHOBATES*
CATESBEIANUS (Shaw, 1802) (ANURA: RANIDAE), IN BRAZIL

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ABSTRACT

We report new records of *Lithobates catesbeianus* feral populations in Brazil. Data were based on fieldwork, natural history collection records, and literature and electronic database searches. *Lithobates catesbeianus* occurs in 130 municipalities of Brazil, including 55 presented for the first time in this work. Most records are from south and southeastern Brazil in the Atlantic Forest biome with climatic conditions that are favorable to the establishment of bullfrog populations. The wide and possibly expanding distribution of feral *L. catesbeianus* populations in Brazil poses a major conservation challenge and demands research on the invasion patterns.

RESUMO

Nós reportamos novos registros de populações na natureza de *Lithobates catesbeianus* no Brasil. Os dados foram baseados em trabalho de campo, registros de coleções científicas, literatura e bancos de dados eletrônicos. *Lithobates catesbeianus* ocorre em 130 municípios do Brasil, dos quais, 55 são apresentados pela primeira vez neste trabalho. Grande parte dos registros são das regiões sul e sudeste do Brasil, em áreas do bioma Mata Atlântica que apresentam condições climáticas favoráveis ao estabelecimento de populações da espécie. A ampla distribuição da espécie invasora, possivelmente em expansão, se apresenta como um grande desafio para a conservação e mostra a urgente necessidade de pesquisas sobre os padrões da invasão da espécie.

INTRODUCTION

The American bullfrog, *Lithobates catesbeianus* (Shaw, 1802), is native to the eastern United States and Canada but over the last century has been transported around the world to be cultured for human consumption. Bullfrogs are generalist predators, show high fecundity, and are strong competitors in both larval and adult phases (Bury and Whelan, 1987; Boelter and Cechin, 2007; Kaefer et al., 2007) — life history traits typical of successful invasive species (Baker, 1974) — and populations are now established in nearly 40 countries in Africa, Asia, North, Central, and South America, and islands of the Mediterranean, South Pacific and Caribbean (Kraus, 2009). Invasive bullfrog populations have been linked either directly, through predation and competition, or indirectly, by inducing habitat use alteration and reducing feeding activity period or and metamorph sizes, to the decline of native amphibian populations in North America (Kats and Ferrer, 2003). Recently, attention has been devoted to the vector role of this species, which appears to be resistant to diseases that are lethal to other amphibians (Daszak et al., 2004).

In South America, recent publications report the presence of invasive bullfrog populations in Argentina, Brazil, Colombia, Ecuador, Uruguay, and Venezuela (Santos-Barrera et al., 2011). Ficetolla et al. (2007) and Giovanelli et al. (2008) showed that the climatic conditions of the southern Atlantic Forest are favorable to the establishment of bullfrog populations, and during fieldwork in this region we found a high number of previously unknown bullfrog populations. Here, we present a new compilation of records of *Lithobates catesbeianus* in Brazil based on our samples, specimens deposited in Brazilian natural history collections, and the literature.

MATERIALS AND METHODS

We searched for bullfrogs at 90 sampling sites, both natural (e.g., ponds, streams) and human-made (e.g., dams), in 10 municipalities (Agudo, Blumenau, Chapecó, Dona Francisca, Guatambu, Indaial, Ivorá, Nova Erechim, Nova Palma, Pinhalzinho) in the states of Rio Grande do Sul and Santa Catarina, southern Brazil. Each site was marked using a GPS and visited at least three times in spring 2009 and spring and summer of 2010 to search for bullfrog egg clutches, tadpoles, and adults. We also conducted occasional surveys in localities of the states of Goiás, Paraná, Rio Grande do Sul, and Santa Catarina.

We complemented our own data with Brazilian *Lithobates catesbeianus* records obtained from published papers, internet databases, and unpublished dissertations and theses. Additionally, we queried curators of scientific collections regarding their bullfrog holdings (Appendix I). We also received “expert information”, i.e. unpublished data from professors and PhD students. Often, we found multiple records in the same municipality. We standardized the data using municipalities as geographical units.

RESULTS

We obtained records of *Lithobates catesbeianus* in 130 Brazilian municipalities; of those, 55 are presented for the first time in this work (Table 1). We also obtained records of bullfrogs published after the most recent compilation by Giovanelli et al. (2008; Figure 1). Approximately 62% of the municipalities are in southern Brazil and 25% are in southeastern Brazil. The states of Rio Grande do Sul and Santa Catarina, southern Brazil, had the most records: 52 and 20 respectively. Northeastern and mid-western Brazil have just seven and nine records, respectively, and just one population is known in the northern region.

DISCUSSION

The increase in the number of known bullfrog localities is immediately evident in Figure 1. The number of municipalities with bullfrog records has doubled in the four years since the last compilation by Giovanelli et al. (2008). Most new records are from south and southeastern Brazil in the Atlantic Forest biome with climatic conditions that are favorable to the establishment of bullfrog populations (Ficetolla et al., 2007; Giovanelli et al., 2008). These are the same regions where we conducted field surveys and other researchers interested in bullfrog biology or bullfrog farming are located. This regional survey bias, combined with the lack of precise historical records, makes it difficult to assess whether the number of bullfrog populations has actually increased since the compilation by Giovanelli et al. (2008). We suspect that the species distribution is greater than we report, at least in Rio Grande do Sul and Santa Catarina states where we have focused our fieldwork. Most residents we interviewed claimed not to know that the bullfrog is non-native and some reported that they often introduce tadpoles to ponds where they raise fishes, while others reported that they had tried to rescue bullfrog tadpoles by transporting them from drying ponds to other water bodies.

Bullfrog farming began in Brazil in 1935 in Rio de Janeiro state and was promoted by the government beginning in the 1940s when tadpoles were donated to be reared even on small properties (Vizotto, 1984; Agostinho, 2003). Scientific advances in aquaculture research in the 1970s were followed by the increase of bullfrog farming in the 1980s. In the early 1990s, it was estimated that there were 2000 bullfrog farms in Brazil (Lima and Agostinho, 2003), but the way the activity was structured was not profitable and several farms closed. This resulted in bullfrogs being released or abandoned, which is the most common beginning of bullfrog invasions, both in Brazil and other countries around the world.

The great distributional potential of *Lithobates catesbeianus* in South America is widely recognized (Ficettola et al., 2007; Giovanelli et al., 2008), given the fast reproductive potential in the subtropical region (Kaefer et al., 2007) and the large variety of potential prey in invaded sites (Boelter and Cechin, 2007; Silva et al., 2009). Scientists are united in calling for stronger policies and control programs to prevent, constrain, and eradicate bullfrog invasions in South America (Pereyra et al., 2006; Boelter and Cechin, 2007; Laufer et al., 2008; Silva et al., 2011), but increasing market pressures are a challenge to any conservation initiative. If in the past bullfrog farming was unprofitable, the strong current global demand for frog legs and improvements in farm structure and technology have made bullfrog farming economically viable (De Bernardi and Alderete, 1999; Warkentin et al., 2007). We believe that frog leg production per se is not necessarily problematic, and the potential economic benefits of bullfrog farming must not be ignored. Instead, the problem lies in the absence of adequate biological and legal safeguards to prevent bullfrog escape or release.

The extensive and possibly expanding distribution of feral *Lithobates catesbeianus* populations in Brazil poses a major conservation challenge, and a lengthy list of research questions must be answered in order to meet it. Data are lacking on particular invasion histories, population densities, dispersal rates, spatial distribution at refined scales, infectious disease incidence, or the impact of invasive bullfrogs on natural ecosystems—especially native frog species, which may already be declining and are expected to be most negatively impacted by invasive anurans. These questions are inextricably linked to problems of public education, economics, and legal regulations, all of which must be addressed to resolve the problem of invasive bullfrogs.

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APPENDIX I - List of natural history collections consulted for *Lithobates catesbeianus* records: Coleção Célio F. B. Haddad, Universidade Estadual Paulista, Campus Rio Claro; Coleção de Amphibia da Universidade Federal da Bahia; Coleção de Anfíbios da Universidade de Passo Fundo; Coleção de Anfíbios da Universidade Federal do Rio de Janeiro; Coleção de Anfíbios do Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul; Coleção de Anfíbios do Museu de Zoologia da Universidade Estadual de Campinas; Coleção de Anfíbios e Répteis do Instituto Nacional de Pesquisas da Amazônia; Coleção de Anfíbios Museu de Biologia Professor Mello Leitão; Coleção de Anfíbios, Universidade Estadual Paulista, Campus São José do Rio Preto; Coleção de Herpetofauna do Museu de Zoologia, Universidade Estadual de Londrina; Coleção de Herpetologia da Universidade Federal de Santa Maria; Coleção de Herpetologia da Universidade Federal do Ceará; Coleção de Coleção de Referência da Herpetofauna de Rondônia, Fundação Universidade de Rondônia; Coleção de Vertebrados do Museu de Zoologia João Moojen de Oliveira, Universidade Federal de Viçosa; Coleção Herpetológica Alphonse Richard Hoge, Instituto Butantan; Coleção Herpetológica da Universidade Federal de Juíz de Fora; Coleção Herpetológica da Universidade Federal do Acre; Coleção Herpetológica da Universidade Federal do Rio Grande do Sul; Coleção Zoológica da Universidade Federal de Goiás.

TABLE 1 - Occurrence points of *Lithobates catesbeianus* in Brazil. Coordinates are presented in decimal degrees.

Municipality	State	Biome	Longitude	Latitude	Source
Águas Mornas	Santa Catarina	Atlantic Rainforest	-48.823600	-27.693900	Present study (MCT 8172)
Agudo	Rio Grande do Sul	Atlantic Rainforest	-53.249920	-29.641570	apud.in Giovanelli et al. (2008)
			-53.279817	-29.498833	Present study
			-53.279150	-29.482066	Present study
Alegre	Espírito Santo	Atlantic Rainforest	-41.533100	-20.763600	Present study
Anápolis	Goiás	Cerrado	-48.952800	-16.326700	Present study
Aparecida de Goiânia	Goiás	Cerrado	-49.243900	-16.823300	Present study
Arroio do Tigre	Rio Grande do Sul	Atlantic Rainforest	-53.096130	-29.338650	apud.in Giovanelli et al. (2008)
Barão	Rio Grande do Sul	Atlantic Rainforest	-51.534560	-29.379320	apud.in Giovanelli et al. (2008)
Barracão	Rio Grande do Sul	Atlantic Rainforest	-51.454420	-27.676980	apud.in Giovanelli et al. (2008)
Belém	Pará	Amazon	-48.504400	-1.455800	Marinho, P. V. (pers. Comm.)
Blumenau	Santa Catarina	Atlantic Rainforest	-49.065417	-26.920317	Dallacorte (2010)
			-49.119017	-27.003000	Present study
			-49.111650	-27.036783	Present study
			-49.112483	-27.037283	Present study
			-49.093667	-27.030350	Present study
			-49.086347	-27.053239	Present study
Brasília	Distrito Federal	Cerrado	-48.010556	-16.035270	Rocha-Miranda et al. (2006)
			-48.010556	-16.035270	Present study
			-48.010556	-16.035270	Present study
Caçador	Santa Catarina	Atlantic Rainforest	-51.202085	-26.753441	Lot. C. L. (pers. comm.)
Camanducaia	Minas Gerais	Atlantic Rainforest	-46.144444	-22.755556	Present study (ZUEC 11542)
Campinas	São Paulo	Atlantic Rainforest	-22.811522	-47.063794	Pizzato, L. (pers. comm.)
Campos do Jordão	São Paulo	Atlantic Rainforest	-45.580080	-22.730460	apud.in Giovanelli et al. (2008)
Campos Novos	Santa Catarina	Atlantic Rainforest	-51.248600	-27.383990	Giovanelli et al. (2008)
Candiota	Rio Grande do Sul	Pampa	-53.683131	-31.481959	Bernardo-Silva, J. (pers. comm.)
Canela	Rio Grande do Sul	Atlantic Rainforest	-50.801870	-29.359920	apud.in Giovanelli et al. (2008)
Caratinga	Minas Gerais	Atlantic Rainforest	-42.140000	-19.790000	apud.in Giovanelli et al. (2008)
Catiporã	Rio Grande do Sul	Atlantic Rainforest	-51.700000	-29.016700	Present study (CFBH 20120, UFRGS 3926)
Caxias do Sul	Rio Grande do Sul	Atlantic Rainforest	-51.167540	-29.178370	apud.in Giovanelli et al. (2008)
Chapecó	Santa Catarina	Atlantic Rainforest	-52.619389	-27.185667	Lucas and Fortes (2008)
			-52.664667	-27.144917	Present study
			-52.652933	-27.143417	Present study

			-52.655833	-27.141717	Present study
Cocal de Telha	Piauí	Caatinga	-41.983330	-4.533330	apud.in Giovanelli et al. (2008)
Coimbra	Minas Gerais	Atlantic Rainforest	-42.800000	-20.866700	Silva et al. (2007)
Coronel Barros	Rio Grande do Sul	Atlantic Rainforest	-54.072797	-28.381386	Present study (UFRGS 4855, 4867)
Corupá	Santa Catarina	Atlantic Rainforest	-49.281810	-26.432660	Giovanelli et al. (2008)
Cotia	São Paulo	Atlantic Rainforest	-46.952450	-23.744130	apud.in Giovanelli et al. (2008)
Curitiba	Paraná	Atlantic Rainforest	-49.433333	-25.716667	apud. in Conte (2010)
Derrubadas	Rio Grande do Sul	Atlantic Rainforest	-53.855300	-27.266270	apud.in Giovanelli et al. (2008)
Dois Lajeados	Rio Grande do Sul	Atlantic Rainforest	-51.850000	-28.983300	Present study (UFRGS 2585)
Dom Feliciano	Rio Grande do Sul	Atlantic Rainforest	-52.110660	-30.697850	apud.in Giovanelli et al. (2008)
Dona Francisca	Rio Grande do Sul	Atlantic Rainforest	-53.358980	-29.626260	apud.in Giovanelli et al. (2008)
			-53.353367	-29.611983	Present study
			-53.353517	-29.611850	Present study
Dona Francisca	Rio Grande do Sul	Atlantic Rainforest	-53.377017	-29.593650	Present study
			-53.336350	-29.612167	Present study
			-53.338600	-29.607517	Present study
			-53.344383	-29.570567	Present study
Duque de Caxias	Rio de Janeiro	Atlantic Rainforest	-43.304460	-22.582420	apud.in Giovanelli et al. (2008)
Embu	São Paulo	Atlantic Rainforest	-46.831910	-23.236399	Giovanelli et al. (2008)
Encantado	Rio Grande do Sul	Atlantic Rainforest	-51.879890	-29.239840	apud.in Giovanelli et al. (2008)
Entre Rios do Sul	Rio Grande do Sul	Pampa	-52.732953	-27.528570	Present study (UFRGS 2555)
Erechim	Rio Grande do Sul	Atlantic Rainforest	-52.268920	-27.629560	apud.in Giovanelli et al. (2008)
Estrela	Rio Grande do Sul	Atlantic Rainforest	-50.980020	-28.100020	apud.in Giovanelli et al. (2008)
Estrela Velha	Rio Grande do Sul	Atlantic Rainforest	-53.153680	-29.172250	apud.in Giovanelli et al. (2008)
Faxinal do Soturno	Rio Grande do Sul	Atlantic Rainforest	-53.426750	-29.572950	Present study
			-53.426683	-29.573467	Present study
			-53.423433	-29.566400	Present study
			-53.423100	-29.580000	Present study
			-53.398817	-29.530483	Present study
			-53.398383	-29.530467	Present study
Fazenda Rio Grande	Paraná	Atlantic Rainforest	-49.250000	-25.616667	Conte and Rossa-Feres (2007)
Fortaleza	Ceará	Caatinga	-38.500000	-3.716700	Pacelli, G. (pers. comm.)
Francisco Beltrão	Paraná	Atlantic Rainforest	-53.099060	-26.064956	Present study
Frederico Westphalen	Rio Grande do Sul	Atlantic Rainforest	-53.400000	-27.366700	Present study (MCT 7469-7473)
Goiânia	Goiás	Cerrado	-49.266700	-16.666700	Present study
Gravataí	Rio Grande do Sul	Atlantic Rainforest	-50.990000	-29.950000	apud.in Giovanelli et al. (2008)
Guaíba	Rio Grande do Sul	Atlantic Rainforest	-51.320000	-30.110000	apud.in Giovanelli et al. (2008)

Guarapuava	Paraná	Atlantic Rainforest	-51.450000	-25.383300	Present study (ZUEC 16841)
Guatambu	Santa Catarina	Atlantic Rainforest	-52.779806	-27.088861	Lucas and Fortes (2008)
			-52.752267	-27.139967	Present study
			-52.751867	-27.163000	Present study
Hidrolândia	Goiás	Cerrado	-49.050000	-17.266700	Present study
Ibarama	Rio Grande do Sul	Atlantic Rainforest	-53.126920	-29.421140	apud.in Giovanelli et al. (2008)
Igrejinha	Rio Grande do Sul	Atlantic Rainforest	-50.800000	-29.580000	Giovanelli et al. (2008)
Indaial	Santa Catarina	Atlantic Rainforest	-49.255867	-26.931817	Present study
			-49.266217	-26.946867	Present study
			-49.265700	-26.946800	Present study
			-49.265217	-26.947800	Present study
Iporanga	São Paulo	Atlantic Rainforest	-48.583300	-24.583300	Present study (ZUEC 16905)
Ipuauçu	Santa Catarina	Atlantic Rainforest	-52.408060	-26.570670	Giovanelli et al. (2008)
Itarana	Espírito Santo	Atlantic Rainforest	-40.875278	-19.873889	Present study (MBML 5587)
Itatiaia	Minas Gerais	Atlantic Rainforest	-43.583300	-20.500000	Present study (CFBH-T 2674)
Ivorá	Rio Grande do Sul	Atlantic Rainforest	-53.527300	-29.482417	Present study
			-53.530467	-29.498517	Present study
			-53.530467	-29.492033	Present study
Jaraguá do Sul	Santa Catarina	Atlantic Rainforest	-49.100000	-26.480000	apud.in Giovanelli et al. (2008)
Joaçaba	Santa Catarina	Atlantic Rainforest	-51.524344	-27.158861	Present study
			-51.588928	27.160858	Present study
Lages	Santa Catarina	Atlantic Rainforest	-50.316700	-27.800000	Present study (MCT 10276)
Lajeado	Rio Grande do Sul	Atlantic Rainforest	-51.126920	-29.421140	apud.in Giovanelli et al. (2008)
Lebon Régis	Santa Catarina	Atlantic Rainforest	-50.666667	-26.853611	Lingnau (2009)
Macaíba	Rio Grande do Norte	Caatinga	-35.350000	-5.850000	Instituto Hórus (2011)
Maceió	Alagoas	Atlantic Rainforest	-35.745130	-9.625540	apud.in Giovanelli et al. (2008)
Manhuaçu	Minas Gerais	Atlantic Rainforest	-42.137461	-20.210497	Silva and Filho (2009)
Marau	Rio Grande do Sul	Atlantic Rainforest	-52.203853	-28.446708	Present study (CAUPF 1828-1834)
Marília	São Paulo	Cerrado	-49.945800	-22.213900	Giovanelli et al. (2008)
Maringá	Paraná	Atlantic Rainforest	-51.937225	-23.427731	Present study (CFBH 17180 - 17183, 17197-17198, 17227)
Mato Castelhana	Rio Grande do Sul	Atlantic Rainforest	-52.187369	-28.278672	Present study (CAUPF 1835-1844, 1853-1858)
Muçum	Rio Grande do Sul	Atlantic Rainforest	-51.869310	-29.165000	apud.in Giovanelli et al. (2008)
Muitos Capões	Rio Grande do Sul	Atlantic Rainforest	-51.183300	-28.316700	Gedoz, A. A. (pers. comm.)
Nonoai	Rio Grande do Sul	Atlantic Rainforest	-52.772433	-27.363242	Present study (UFRGS 2581, 4341)
Nova Erechim	Santa Catarina	Atlantic Rainforest	-52.899817	-26.869667	Present study
			-52.921350	-26.886633	Present study

		Atlantic Rainforest	-52.925308	-26.881517	Present study
Nova Palma	Rio Grande do Sul	Atlantic Rainforest	-53.472230	-29.471450	apud.in Giovanelli et al. (2008)
		Atlantic Rainforest	-53.480000	-29.475083	Present study
		Atlantic Rainforest	-53.486400	-29.474150	Present study
Nova Petrópolis	Rio Grande do Sul	Atlantic Rainforest	-51.140000	-29.360000	apud.in Giovanelli et al. (2008)
Nova Roma do Sul	Rio Grande do Sul	Atlantic Rainforest	-51.406670	-28.999720	apud.in Giovanelli et al. (2008)
Palmas	Paraná	Atlantic Rainforest	-52.000000	-26.500000	Camargo-Neto, A (pers. Comm.)
Pariquera-Açu	São Paulo	Atlantic Rainforest	-47.883300	-24.716700	Giovanelli et al. (2008)
Parnaíba	Mato Grosso do Sul	Cerrado	-51.195836	-19.671744	Present study (CFBH 20116)
Parnamirim	Rio Grande do Norte	Atlantic Rainforest	-35.260289	-5.906186	Instituto Hórus (2011)
Passo Fundo	Rio Grande do Sul	Atlantic Rainforest	-52.410000	-28.260000	Present study (CAUPF 2303)
Passos Maia	Santa Catarina	Atlantic Rainforest	-51.900000	-26.783333	Conte (2010)
Paulista	Pernambuco	Atlantic Rainforest	-34.883300	-7.950000	Instituto Hórus (2011)
Pedro de Toledo	São Paulo	Atlantic Rainforest	-47.231370	-24.287260	Giovanelli et al. (2008)
Peruíbe	São Paulo	Atlantic Rainforest	-46.994378	-24.316311	Present study (CFBH 12711)
Pinhalzinho	Santa Catarina	Atlantic Rainforest	-52.970167	-26.832067	Present study
			-52.969250	-26.831650	Present study
			-52.952550	-26.836350	Present study
			-52.952450	-25.839400	Present study
			-52.942600	-26.815383	Present study
			-52.938317	-26.812867	Present study
Piracicaba	São Paulo	Atlantic Forest/ Cerrado	-47.640000	-22.710000	apud.in Giovanelli et al. (2008)
Pomerode	Santa Catarina	Atlantic Rainforest	-49.175686	-26.740608	Present study
Ponte Nova	Minas Gerais	Atlantic Rainforest	-42.925003	-20.371067	Camargo-Filho et al. (2008)
Ponte Serrada	Santa Catarina	Atlantic Rainforest	-52.020528	-26.870203	Conte (2010)
Porto Alegre	Rio Grande do Sul	Atlantic Rainforest	-51.220000	-30.040000	apud.in Giovanelli et al. (2008)
Quatro Barras	Paraná	Atlantic Rainforest	-49.010140	-25.310060	Present study (CFBH 18142)
			-49.001490	-25.287120	Present study (CFBH 18150 - 18153)
Rio Claro	São Paulo	Atlantic Rainforest/ Cerrado	-47.571170	-22.354710	Giovanelli et al. (2008)
Rio do Sul	Santa Catarina	Atlantic Rainforest	-49.641967	-27.215347	Present study
Rio Pardo	Rio Grande do Sul	Atlantic Rainforest	-52.371653	-29.984625	Present study
Roca Sales	Rio Grande do Sul	Atlantic Rainforest	-51.871406	-29.289733	Present study (MCT 8435)
Salvador	Bahia	Atlantic Rainforest	-38.509344	-12.971672	Instituto Hórus (2011), Present study (UFBA, 4451)
Santa Cruz do Sul	Rio Grande do Sul	Atlantic Rainforest	-52.433710	-29.715770	apud.in Giovanelli et al. (2008)
Santa Maria	Rio Grande do Sul	Atlantic Rainforest/ Pampa	-53.828530	-29.695240	apud.in Giovanelli et al. (2008)
			-53.700000	-29.700000	Santos et al. (2008)
Santa Tereza	Rio Grande do Sul	Atlantic Rainforest	-51.745860	-29.168810	apud.in Giovanelli et al. (2008)

Santo Antônio do Descoberto	Goiás	Cerrado	-48.260300	-15.969680	Giovanelli et al. (2008)
São João do Polêsine	Rio Grande do Sul	Atlantic Rainforest	-53.443017	-29.621558	Present study
São José do Rio Preto	São Paulo	Cerrado	-49.379400	-20.819700	Giovanelli et al. (2008)
São José dos Pinhais	Paraná	Atlantic Rainforest	-49.195725	-25.530808	apud in Giovanelli et al. (2008)
São Luís do Paraitinga	São Paulo	Atlantic Rainforest	-45.133000	-23.346000	Giovanelli et al. (2008)
São Paulo	São Paulo	Atlantic Rainforest	-46.642900	-23.776140	Giovanelli et al. (2008)
Senador Canedo	Goiás	Cerrado	-49.083300	-16.716700	Present study
Serafina Correa	Rio Grande do Sul	Atlantic Rainforest	-51.932167	-28.712392	Present study (UFRGS 3445-3446, 3457-3458)
Silva Jardim	Rio de Janeiro	Atlantic Rainforest	-42.393500	-22.655589	Potsch, S. (pers. comm.)
Silvânia	Goiás	Cerrado	-48.610286	-16.665144	Bastos, R. (pers. comm.)
Suzano	São Paulo	Atlantic Rainforest	-46.299920	-23.569800	Giovanelli et al. (2008)
Tapes	Rio Grande do Sul	Pampa	-51.396614	-30.674347	Present study (UFRGS 3171)
Taquari	Rio Grande do Sul	Atlantic Rainforest	-51.860260	-29.801090	apud.in Giovanelli et al. (2008)
Telêmaco Borba	Paraná	Atlantic Rainforest	-50.583333	-24.283333	Machado (2004)
Tenente Portela	Rio Grande do Sul	Atlantic Rainforest	-53.760150	-27.375910	apud.in Giovanelli et al. (2008)
Teresópolis	Rio de Janeiro	Atlantic Rainforest	-42.966378	-22.412578	Potsch, S. (pers. comm.)
Torres	Rio Grande do Sul	Atlantic Rainforest	-49.755600	-29.348210	apud.in Giovanelli et al. (2008)
Triunfo	Rio Grande do Sul	Pampa	-51.718075	-29.942667	Present study (MCT 5614)
Tunas	Rio Grande do sul	Atlantic Rainforest	-52.991339	-29.126781	Bernardo-Silva, J., (pers. comm.)
Ubatuba	São Paulo	Atlantic Rainforest	-45.132530	-23.497280	Giovanelli et al. (2008)
Uberlândia	Minas Gerais	Cerrado	-48.279970	-18.900010	apud.in Giovanelli et al. (2008)
Varginha	Minas Gerais	Atlantic Rainforest	-45.100000	-22.333300	Present study (CFBH-T 7514)
Veranópolis	Rio Grande do Sul	Atlantic Rainforest	-51.560000	-28.940000	apud.in Giovanelli et al. (2008)
Viamão	Rio Grande do Sul	Atlantic Rainforest	-50.980000	-30.090000	apud.in Giovanelli et al. (2008)
Viçosa	Minas Gerais	Atlantic Rainforest	-42.882219	-20.754200	apud.in Giovanelli et al. (2008); Silva et al. (2009)
Vieiras	Minas Gerais	Atlantic Rainforest	-42.294728	-20.961692	Silva and Filho (2009)
Vila Flores	Rio Grande do Sul	Atlantic Rainforest	-51.551892	-28.868928	Present study (MCT 9933)
Vitória	Espírito Santo	Atlantic Rainforest	-40.412720	-20.331070	apud.in Giovanelli et al. (2008)
Xaxim	Santa Catarina	Atlantic Rainforest	-52.534097	-26.961517	Present study (MCT 9768)

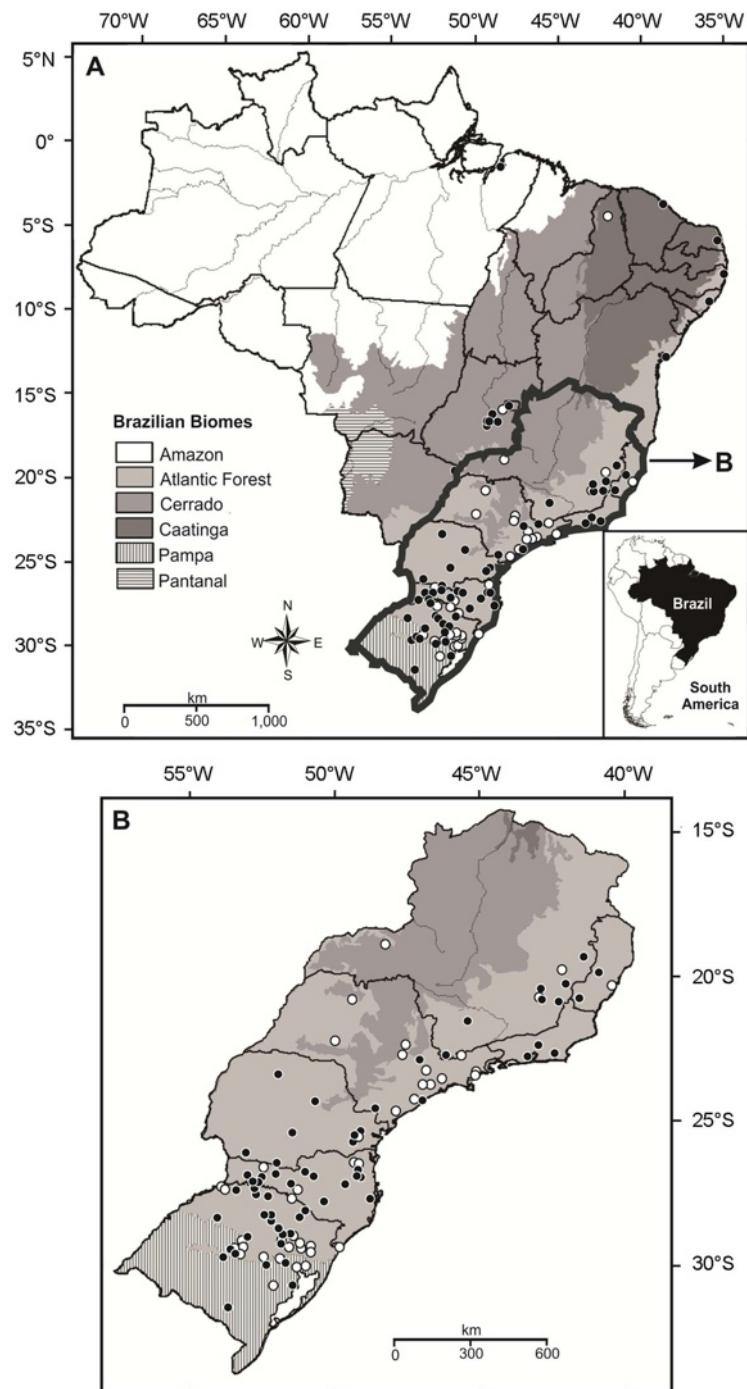


FIGURE 1 - Spatial distribution of *Lithobates catesbeianus* invasive populations in Brazil (A). In B, the species distribution in south and southeastern regions appear in detail. White circles represent municipalities in which the bullfrog presence was already known in 2008 (Giovanelli et al. 2008), and the black circles represent the subsequent records reported herein.

5. Capítulo II

Fine-scale environmental descriptors predict invasive *Lithobates catesbeianus* distribution in the Brazilian Atlantic Forest

Fine-scale environmental descriptors predict invasive *Lithobates catesbeianus* distribution in the Brazilian Atlantic Forest

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Summary

1. *Lithobates catesbeianus*, the American bullfrog, is one of the world's 100 worst alien species. The climatic conditions of the Atlantic Forest of southern Brazil are highly suitable for this species and feral populations have become established at many localities, including protected areas. Factors governing distribution patterns are unknown and there is an urgent need to identify environmental factors that could be useful to prevent further expansion.
2. We conducted surveys at 90 waterbodies in three invaded areas of Atlantic Forest, collecting data on *L. catesbeianus* abundance and breeding to assess the importance of spatial and environmental descriptors using robust variation partitioning techniques.
3. Waterbody descriptors were the primary filters for *L. catesbeianus* abundance and predicted the probability that a site will be used for breeding. Waterbody depth was the most important filter and was highly correlated with artificial ponds, presence of fish, and greater waterbody area and permanence. Landscape features were secondary, with greater abundances and breeding probabilities when surrounding areas were used for grazing and included artificial ponds and streams. Spatial structure was relevant only at the largest scale studied, explaining differences in abundance between areas.
4. Our results point to management actions that can be taken at the local scale. By preventing the construction of artificial ponds and/or limiting their maximum depth to < 55.5 cm, it is likely that of invasive *L. catesbeianus* populations can be significantly impeded.
5. **Synthesis and applications.** The invasive species *Lithobates catesbeianus* is fully established and expanding in the Atlantic Forest biome of Brazil. Despite high

climatic suitability and increasing number of population records, the spatial distribution of *L. catesbeianus* and associated landscape and habitat features have not been studied. We tested which environmental factors explain the distribution of *L. catesbeianus* in the southern Atlantic Forest at regional and local scales using variation partitioning approaches. Waterbody depth was the most important predictor of both abundance and breeding, with a threshold at 55.5 cm maximum depth. By preventing the construction of artificial ponds and/or limiting their maximum depth, managers could limit population sizes and impede the establishment of invasive *L. catesbeianus*.

Keywords: alien species, Amphibia, abundance, biological invasion, breeding, bullfrog, landscape, management, spatial structure, waterbodies

Introduction

In order for invasive populations to become established in a new landscape, they must overcome myriad environmental barriers that directly affect survival, reproduction, and dispersal and operate at spatial scales from regions to microhabitats (Richardson *et al.* 2000; Blackburn *et al.* 2011). Regional climatic suitability functions as a broad-scale barrier (Richardson & Thuiller 2007); however, even in regions with highly suitable climates, establishment of invasive populations may not occur due to landscape and fine-scale habitat and microhabitat characteristics, which function as a second class of barriers (Hobbs & Huenneke 1992; Von Holle & Simberloff 2005). Finally, biotic interactions with native species can filter alien invasions by either promoting or preventing establishment (Fridley *et al.* 2007), and all of these barriers can also act at different stages of the invasion process (Blackburn *et al.* 2011).

Prediction of invasive species distribution is essential for linked research and conservation initiatives, and improved understanding of the factors governing invasive species distributions is urgently needed in some cases. The American bullfrog invasion of the Atlantic Forest is one such case. With feral populations established in nearly 40 countries (Kraus 2009), this species, *Lithobates catesbeianus* (Shaw 1802), is considered one of the 100 worst invasive species of the world (Lever 2003). It is native to eastern North America where it occurs in a wide range of climatic conditions, which is one of the reasons for its successful establishment around the world (Ficetola, Thuiller & Miaud 2007). Invasive bullfrogs have been implicated in the decline of native amphibians in North America (Adams & Pearl 2003; Kats & Ferrer 2003;) and the global spread of the chytrid fungus *Batrachochytrium dendrobatidis*, which causes the disease chytridiomycosis and is associated with amphibian declines around the world (Garner *et al.* 2006; James *et al.* 2009). *Lithobates catesbeianus* is widespread in Brazil, but records

are primarily concentrated in the Atlantic Forest (Giovanelli, Haddad & Alexandrino 2008; Both *et al.* 2011), a global amphibian hotspot already under strong anthropogenic pressure (Ribeiro *et al.* 2009).

Several niche models of the potential distribution of *L. catesbeianus* in Brazil agree on the high suitability of the Atlantic Forest. Ficetola, Thuiller & Miaud (2007) used current climatic descriptors and the human footprint to model the species distribution and showed that southern and southeastern regions of the Brazilian Atlantic Forest are especially favourable. Giovanelli, Haddad & Alexandrino (2008) used climatic and topographic descriptors to develop a distribution model specific to Brazil and predicted a similar distribution. Models based on current and future climatic conditions derived from alternative green-house gas emission scenarios agreed that Atlantic Forest regions currently present the best conditions for *L. catesbeianus* populations and predicted that the southern portions of the biome will remain most suitable in the future (Nori *et al.* 2011). Of special concern is the conclusion by Nori *et al.* (2011) and Loyola *et al.* (2012) that climate change is likely to favour invasion of protected areas intended as refuges for native Atlantic Forest communities.

While niche modelling studies warn of the high potential for future invasions of *L. catesbeianus* into protected areas, records of feral populations within Atlantic Forest conservation units have already been reported (Lucas & Fortes 2008; Iop *et al.* 2011), and we have observed several more (C.B. unpublished data). Despite the alerts about the high climatic suitability of the biome and increasing number of population records (Both *et al.*, 2011), no study has evaluated the spatial distribution of *L. catesbeianus* in the region or the landscape and habitat features associated with the observed distribution.

In this study we evaluate the roles of spatial structure, landscape, and waterbodies as factors in the distribution of *L. catesbeianus* in three different areas of the Atlantic Forest biome of southern Brazil, all of which are highly suitable to population establishment. We model both (i) abundance of post-metamorphic individuals and (ii) breeding site choice. In the first case, we examine the general distribution of the species, including temporary occurrences by dispersing individuals. In the second case, we consider only sites where *L. catesbeianus* is effectively established, which can be viewed as management units for aquatic breeding anurans (Hagman & Shine 2006). We expect that both spatial and environmental factors predict the distribution patterns and hypothesise that waterbody descriptors and surrounding landscape features are most important, rather than space itself. By determining the relative importance of environmental descriptors in explaining the distribution of invasive *L. catesbeianus* in the Atlantic Forest, we aim to identify measures that could prevent the invasion of priority conservation areas.

Materials and Methods

Study Areas

The study was conducted in the Atlantic Forest of central Rio Grande do Sul (area A1) state and western (A2) and eastern (A3) Santa Catarina state (Fig. 1). All areas are highly suitable for *L. catesbeianus* establishment (Giovanelli, Haddad & Alexandrino 2008; Nori *et al.* 2011) and present the greatest density of Brazilian records (Both *et al.* 2011). We do not know the precise invasion histories of each region, but according to interviews with local residents they all date from at least 10 years prior to our study.

Study area A1 comprises the municipalities of Agudo, Dona Francisca, Ivorá and Nova Palma. In this area, the vegetation is characterised by Seasonal Deciduous Forest (IBGE 2004). A2 includes Chapecó, Guatambu, Nova Erechim and Pinhalzinho municipalities. In this area, both Seasonal Deciduous Forest and Mixed Ombrophilous Forest (IBGE 2004; Lucas & Fortes 2008) occur, with *Araucaria angustifolia* being frequent at all surveyed sites. A3 includes Blumenau and Indaial and is characterised by Dense Ombrophilous Forest (IBGE 2004).

The Atlantic Forest is highly fragmented and currently comprises less than 8% of the original forest (Fundação SOS Mata Atlântica/INPE 2009). The general land use is farming (e.g. soy, corn, tobacco) and cattle grazing. A small proportion of land is used for recreation, with landscapes composed of small gardens and/or pools surrounded by forest. Larger forest fragments occur in protected areas. In A1, Parque Estadual da Quarta Colonia protects 1,847.90 ha along the border of the Dona Francisca hydroelectric dam. Floresta Nacional de Chapecó, with 1,606.3 ha divided in two areas, occurs in A2, and Parque Nacional da Serra do Itajaí, with 57,374 ha, is located within A3.

Sampling

During initial surveys in spring 2009, a period of high anuran reproductive activity, we selected waterbodies for subsequent sampling, including natural marshes, ponds, stream sections with low water flow, and artificial ponds. We returned in 2010 to sample all sites twice, once in February/March (late summer) and again in October/November (spring). Sampling periods overlap with times of intense reproductive activity by *L. catesbeianus* in Brazil (Kaefer, Boelter & Cechin 2007) and follows the general activity

pattern of native communities (Both *et al.* 2008; Santos *et al.* 2008). We sampled 90 waterbodies: 32 in A1, 30 in A2 and 28 in A3 (see *Appendix S1*). We actively searched for *L. catesbeianus* at all sites and considered the occurrence of calling males, eggs, and/or tadpoles as evidence of breeding. In each area, half of the waterbodies presented evidence of *L. catesbeianus* breeding and half did not, with sites selected as close to each other as possible to strengthen comparisons. This design avoids high spatial clustering of species occurrences and focuses on the environmental variation.

On any given day we sampled 4–6 sites for eggs and tadpoles during the day and post-metamorphic individuals at night. To search for evidence of breeding, we sampled tadpoles in distinct microhabitats (e.g., near the edge of the waterbody, with and without vegetation; deep water near the centre of the waterbody, with and without vegetation) with dipnets (40 x 30 cm, 2 mm mesh). Sampling effort was proportional to the pond size and heterogeneity (Shaffer *et al.* 1994). A minimum of five dipnet sweeps were conducted in each microhabitat. All samples were taken between 9 a.m. and 19 p.m. We also conducted visual searches for egg clutches, which differ markedly from those of all native species in these areas. Visual and acoustic searches for post-metamorphic individuals around the perimeters of breeding sites began 30 min after sunset. Sampling effort was proportional to water body size and complexity (Scott & Woodward 1994).

Landscape and waterbody descriptors

To characterise the surrounding landscape at each waterbody, we measured the distance to the closest forest fragment and road (all roads were lightly used secondary roads) and all waterbodies within a 400 m radius of each site. In the same radius, we classified land use as grazing, crops, protected (nature reserves), or leisure (private properties without

economic activities). Waterbody and land use types were scored as present-absent, often with different types co-occurring around the same sampling site. The availability of waterbodies is closely related to land use, given that artificial ponds are often constructed at grazing and leisure sites, while streams and marshes often occur in preserved sites. We performed a correspondence analysis using the frequency of occurrence of the binary landscape descriptors using the CA function of the FactoMiner R package (Mazzet & Husson 2012). Besides waterbody distances to fragments and roads distances, the first three coordinates, accounting for 57% of total variation, were used as landscape descriptors in subsequent analyses. The scatterplot of the two first land use descriptors is shown in Fig. 2. The correspondence analysis and all other analyses in the study were performed using R 2.15.0 (R Development Core Team 2012).

We measured the area and depth of each waterbody in each collection event. We measured the depth of each microhabitat sampled for tadpoles and recorded the mean, maximum, and minimum for all microhabitats at each site. The ratio of the areas measured at the two sampling events (summer area/spring area) was used as an estimate of pond permanence. We recorded the richness of hydrophyte morphotypes (floating and emergent macrophytes) and visually estimated hydrophyte coverage in three classes: < 30%, 30–60 % and > 60%. Fish presence was determined through visual surveys, dipnet captures, and interviews with land owners. Descriptive statistics for quantitative waterbody descriptors are presented in Table 1.

All quantitative descriptors were expanded into second and third order monomials, which were used to control for nonlinear relationships between habitat descriptors and distribution data. The complete matrix of environmental descriptors, E, included (i) the land use factors from the correspondence analysis and the distances from

roads and fragments and respective monomials, and (ii) all pond descriptors and respective monomials.

Spatial structure

The spatial structure of sites in this study has two major scales: between areas (A1, A2, and A3) and within areas. Within areas, we intentionally selected spatially proximate pairs of breeding and non-breeding sites to allow better characterisation of site selection. As such, we expect breeding sites spatial arrangement to show low structured variation. In contrast, spatially structured abundances at both between and within area scales could occur in relation to either the space itself or spatially structured environmental descriptors (Legendre & Legendre 1998).

We considered two kinds of spatial descriptors in the matrix S: the three large study areas, represented by dummy variables, and Moran's eigenvector maps (MEM). MEMs provide orthogonal spatial variables ranging from broad, inter-area scales to the finest scales derived from geographic coordinates of the sites (Dray, Legendre & Peres-Neto 2006). The spatial arrangement in this study shows a high truncation value (minimum distance connecting all points = 334 km) between study areas. Therefore, we used a nested MEM model (Declerck *et al.* 2011), where MEM variables are blocked within study areas to describe spatial relationships at this scale. We used the R function `create.MEM.model` (Declerck *et al.* 2011) to build the MEM model using a matrix of dummy variables describing the three regions as the connectivity criteria. The nested analysis resulted in seven spatial vectors describing the spatial arrangement of sites within areas.

***Lithobates catesbeianus* distribution**

We quantified the importance of environmental and spatial descriptors for *L. catesbeianus* distribution through variation partitioning based on linear models (Borcard 1992). In this way, we assessed the independent influence of the environmental and spatial descriptors on distribution and controlled for spatially structured environmental variation. To characterise distribution, we used the relative abundance per site, given by the maximum number of post-metamorphic individuals recorded in a single survey.

To model *L. catesbeianus* relative abundance with environmental matrix E, we used a linear model and forward selection of descriptors with the R function `forward.sel` of the “packfor” package (Dray *et al.* 2011). We adopted $p \leq 0.05$ as the cutoff for variable inclusion and selection by double stopping criteria (Blanchet, Legendre & Borcard 2008). The variable inclusion and full model were tested through 999 Monte Carlo permutations. We used the same procedure to model distribution with the spatial matrix S. A third model containing the selected environmental variables and MEMs was then used to estimate the shared variation between matrices E and S. Variation partitioning was evaluated using the `varpart` R function in the package “vegan” (Legendre 2011).

Breeding sites

The 90 waterbodies were classified as either breeding sites (1), where we found *L. catesbeianus* egg clutches, tadpoles, and/or calling males, or non-breeding sites (0), which lacked evidence of reproduction. We used a regression tree to relate the breeding sites to matrices E and S, which is both the most appropriate approach for multinomial distributions and a powerful predictive tool to guide management decisions (De’Ath & Fabricius 2000). The analysis consists of a recursive binary partitioning that allocates

response objects to two homogenous groups in the resulting two nodes (De'Ath 2002). The tree grows by consecutively partitioning nodes using the same or next explanatory variable that reduces leaf impurity, moving forward until the desired size. We used cross-validation to prune the tree ($xval = 100$). The analysis was carried out using the “rpart” package (Therneau & Atkinson 2006).

Results

We observed *L. catesbeianus* in 62 of the 90 sampled waterbodies. Postmetamorphic abundances ranged from 1 to 51 (mean = 7.7, SE = 1.3) and were related to maximum waterbody depth, hydrophyte richness, and landscape factor CA2 (Table 2). Those predictors accounted for 31% of the variation in abundance. The relationship between *L. catesbeianus* abundance, mean depth, and hydrophyte richness was positive, whereas the CA2 axis negatively predicted abundances. Maximum waterbody depth showed the highest coefficient of determination and was positively correlated with artificial ponds ($r = 0.60$), fish presence ($r = 0.57$), area ($r = 0.21$), and permanence ($r = 0.46$).

Lithobates catesbeianus abundance was also predicted by the spatial component. The vector distinguishing A3 from the other two areas was the only spatial descriptor retained in the model (Table 2). Figure 3 shows the distribution of abundances in the three study areas and highlights the low abundances in A3 relative to other areas.

The model combining the selected descriptors of environmental and spatial matrices accounted for 37% of the variation in *L. catesbeianus* distribution. The environmental component alone accounted for 30% of the distribution, while the spatial component explained 6% of the variation. The spatially structured environmental variation explained the remaining 1%. The shared variation between the spatial and

environmental components was exclusively related to the correlation of vector A3 with landscape factor CA2 ($r = 0.43$, $p < 0.05$).

We found evidence of *L. catesbeianus* breeding activity at 44 waterbodies: 16 in A1, 16 in A2, and 12 in A3. Thirty were artificial ponds and 14 were natural ponds. We did not detect any evidence of reproduction in streams. The regression tree analysis indicated that the first factor to increase the probability of *L. catesbeianus* breeding at a site is maximum waterbody depth (Fig. 4). Of the 38 sites < 55.5 cm maximum depth, 35 (92%) were not used for breeding; of the 52 sites > 55.5 cm maximum depth, 41 (79%) were used for breeding. The second split classified sites according to landscape factor CA2. Sites with scores ≥ -19.07 on the CA2 axis (Fig. 2) were used for breeding, with only one exception. Sites with scores > -19.07 on the landscape axis ($n=25$) were further divided into sites with areas > 226.3 m², most of which were used for breeding (10 of 11 sites), and sites with smaller areas, most of which were non-breeding sites (9 of 14). Further splits were not validated.

Discussion

The distribution of *L. catesbeianus* in the southern portion of the Brazilian Atlantic Forest is determined primarily by waterbody factors, with the surrounding landscape and space at a larger scale contributing much less to the explanation of *L. catesbeianus* variation. Local filters also determine the probability that a waterbody is used for breeding, while landscape—surrounding land use and waterbody availability—is a second-order factor.

Lithobates catesbeianus is a highly aquatic species that breeds in lentic water and has tadpoles that can last several years prior to metamorphosis and adults that rarely

stray from waterbodies (Bury & Whelan 1984). Given such life history constraints, it is logical that habitat factors at the scale of waterbodies would be the main predictors of distribution in both native and invaded areas. Waterbody depth was most strongly related to both *L. catesbeianus* abundance and breeding sites. This variable was positively correlated with area, permanence, presence of fish, and artificial ponds, all of which have been associated with invasive *L. catesbeianus* distribution in previous studies (e.g. Bunnell & Zampella 2008; Wang & Li 2009). Consistent with the finding that waterbody depth is the main predictor, our samples included natural ponds without fishes that were sufficiently deep to sustain feral populations. Natural ponds often support high hydrophyte richness, which also positively predicted abundances. Aquatic macrophytes might protect egg clutches and provide refuge for tadpoles and juveniles (Wellborn, Skelly & Werner 1996; Kopp, Wachlewski & Eterovick 2006) but could also support *L. catesbeianus* prey, especially other anurans (Silva *et al.* 2011). In this study we did not find individuals reproducing in streams, but stream breeding was reported in further north in Minas Gerais state (Afonso *et al.* 2010), and the use of disturbed rivers for breeding is also known in invaded sites in United States (Fuller *et al.* 2011).

Given the positive relationship between *L. catesbeianus* and open and disturbed habitats, the extensive and increasing fragmentation of the Atlantic Forest could facilitate population expansion. Roads, streams, and open areas might facilitate colonisation of natural reserves and larger forest fragments, although data on *L. catesbeianus* movement are lacking and our models did not select distance to forest fragment or road as important predictors. Instead, our analyses highlighted the importance of the landscape factors describing surrounding land use and waterbodies. These landscape factors explained little of the variation in distribution, but abundances were greater in grazing areas with nearby artificial ponds, natural ponds, and streams,

which could function as an aquatic network. The second landscape factor (CA2) related to breeding sites in a similar way and also interacted with pond area: aquatic sites of greater area in preserved landscapes were more likely to exhibit *L. catesbeianus* breeding activity. This suggests that if *L. catesbeianus* can reach preserved areas (e.g., via open areas or aquatic networks) it will be able to inhabit them.

Spatial structure was only important at the largest scale studied, with lower *L. catesbeianus* abundances in A3 than the other areas. This spatial variation was partially related to landscape factors. A3 possessed a greater number of preserved or leisure sites with little alteration to the natural landscape. A3 was also the only region characterised by Dense Ombrophilous Forest, and factors related to the native biodiversity, another possible filter (Fridley *et al.* 2007), could also explain this pattern. We did not detect spatial structure at finer scales, which could be due to the paired design and/or the stage of the invasion.

Even as numerous niche modelling studies have warned that the highly suitable climate of the Atlantic Forest places this region in extreme risk for the widespread establishment of feral populations of *L. catesbeianus* (Ficetola, Thuiller & Miaud 2007; Giovanelli, Haddad & Alexandrino 2008; Nori *et al.* 2011), researchers at the Brazilian Agricultural Research Corporation (EMBRAPA) have employed a similar approach to model the best climatic conditions for bullfrog farming (Rodrigues *et al.* 2010). As such, it should come as no surprise that *L. catesbeianus* appears to be in the final stage of the invasion of this region, corresponding to “fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence” (Blackburn *et al.* 2011: 337). Although the effects of invasive *L. catesbeianus* on native Atlantic Forest biodiversity are unknown, there is also no scientific basis for the assertion by some economists that “the

environmental impacts of raniculture in Brazil are taken to be insignificant” (Feix *et al.* 2006:73), and given its apparently expanding distribution (Both *et al.* 2011), its observed and predicted (Loyola *et al.* 2012) ability to successfully invade conservation units, and its potential to have major, irreversible, negative impacts on native biodiversity (Adams & Pearl 2007), the precautionary principle (Kriebel *et al.* 2001) dictates that measures be taken to prevent further expansion, especially into protected areas.

Both climate change and landscape modification are enormous problems that are well beyond the purview of ecosystem managers and regional policy makers. However, our findings point to actions that can be taken at a local scale. We found that waterbody depth was the most important predictor of both *L. catesbeianus* abundance and breeding and detected a strong threshold effect at 55.5 cm maximum depth, whereby most ponds < 55.5 cm were not used for breeding and most ponds > 55.5 cm were used for breeding. Moreover, most waterbodies > 55.5 cm maximum depth were artificial ponds formed by damming small streams. Consequently, by preventing the construction of artificial ponds in and near protected areas and/or limiting their maximum depth to < 55.5 cm, it is likely that managers could impede the establishment of invasive *L. catesbeianus* and limit population size where establishment occurs. A further step that deserves careful consideration is to eliminate or reduce the depth of existing artificial ponds, but we caution that the efficacy of this measure must be studied on a case-by-case basis, with potential benefits weighed against the effects on existing terrestrial and aquatic communities (Adams & Pearl 2007).

Bullfrog farming has great potential as a profitable business that could positively impact the lives of many Brazilians in rural areas and might be less ecologically damaging than alternative agricultural practices like cattle ranching and sugar cane production. However, adequate measures must be taken to prevent escape and the

establishment of feral populations and protect native biodiversity. Although bullfrog farms did not occur in our study areas, bullfrog production in Brazil is expanding (Feix, Abdallah & Figueiredo 2006; Rodrigues *et al.* 2010) and Liu and Li (2009) emphasised the role of bullfrog farms in promoting invasions in China. Liu & Li (2009) also found that elaborate enclosures (e.g. artificial ponds inside brick) that are more effective at preventing escapes significantly reduce the probability of the establishment of feral populations relative to simple enclosures (1.5 m high fences around outdoor waterbodies). As such, bullfrog farms should meet adequate structural requirements to prevent escape by both tadpoles and post-metamorphic individuals and be prohibited in the vicinity of protected areas. Similarly, insofar as escapees require appropriate breeding sites in order to establish feral populations, artificial ponds > 55.5 cm deep should be prohibited in the areas surrounding bullfrog farms.

Although our results provide insights into the invasion of *L. catesbeianus* in the Atlantic Forest of southern Brazil and point to practical measures to be taken by environmental managers and regional policy makers to limit feral population sizes and impede establishment, research is urgently required to address the following major lacunas.

- (i) Biotic filters. In the present study, we examined the roles of abiotic environmental features as factors in the distribution of invasive *L. catesbeianus*, but biotic interactions can also filter alien invasions (Fridley *et al.* 2007). Understanding the relationship between invasive *L. catesbeianus* and native biodiversity will provide crucial insights into potential impacts of bullfrog invasion on Atlantic Forest fauna.
- (ii) Movement. There is a dearth of information on the patterns and distances of invasive *L. catesbeianus* movement. Given the positive relationship between *L. catesbeianus* and open and disturbed habitats, it is likely that individuals use roads and disturbed

areas as dispersal corridors, like invasive *Rhinella marina* (cane toads) in Australia (Brown *et al.* 2006). Knowledge of the distances travelled by individuals is also critical both to predict expansions (Phillips *et al.* 2007) and determine the extent of the artificial pond-free buffer zone that should be maintained around protected areas and bullfrog farms. Dispersal of 7–8 km by invasive *L. catesbeianus* in North America is likely (Schwalbe & Rosen 1999), but studies using modern tracking equipment are lacking for both native and invasive *L. catesbeianus*. Moreover, Brown *et al.* (2006) found that *R. marina* evolved longer legs that allow faster movement and accelerated invasion, and similar adaptations could have increased the speeds and distances travelled by *L. catesbeianus* as well.

(iii) Effects of invasive *L. catesbeianus* on native Atlantic Forest biodiversity. Although the potential negative impacts of invasive *L. catesbeianus* on native species are widely recognised (*e.g.* Kats & Ferrer 2003), evidence of actual impacts in the Atlantic Forest are lacking. Stomach contents of invasive *L. catesbeianus* reveal a diverse diet of small vertebrates and macroinvertebrates (*e.g.* Boelter & Cechin 2007), but predation alone does not necessarily entail effects at the population of community scale. It is notoriously difficult to causally relate observed changes to an invasive species, and harder still to identify the specific pathways responsible for those changes (Shine 2010). Both & Grant (2012) showed that *L. catesbeianus* advertisement calls induce significant changes in the acoustic signals used by *Hypsiboas albomarginatus* frog to attract and assess mates and evaluate rivals, but the effects of those changes on reproductive success and population structure have not been tested.

Lithobates catesbeianus is fully established and expanding in the Atlantic Forest of Brazil. Given the extent of the invasion and the increase of bullfrog farming in Brazil,

it is essential that researchers, law and policy makers, law enforcement, ecosystem managers, non-governmental organisations, and the agricultural industry work together to improve understanding of the processes involved in the invasion and its effects on native biodiversity to implement the most efficient means of reducing negative impacts and preventing further expansion, especially in protected areas.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Geographic coordinates of sampled sites.

Table 1 - Descriptive statistics for quantitative waterbody descriptors from 90 sampled sites in three areas of the Atlantic Forest.

	Mean	SD	Min.	Max.
Distance to the next fragment (m)	151.0	214.2	0.0	660.0
Distance to the next road (m)	350.0	194.1	0.0	985.7
Area (m ²)	771.9	3965.9	7.9	37341.8
Depth (cm)	61.9	30.1	6.0	152.0
Permanence degree (summer area/spring area)	0.6	0.4	0.0	1.0
Hydrophyte richness (morphotypes)	5.7	5.0	0.0	23.0

Table 2 - Results of forward selection of environmental and spatial models of *Lithobates catesbeianus* abundance distribution. R^2 values report the cumulative changes in the coefficient of variation corrected for multiple predictors.

	$R^2_{\text{cum. adjust.}}$	F	p	Sign
Environmental component				
Depth (mean)	0.22	26.03	0.001	+
Hydrophyte richness	0.28	8.13	0.014	+
Landscape - CA2	0.31	5.37	0.017	-
Spatial component				
A3	0.06	7.14	0.007	-



Figure 1 - Location of tree studied areas in South Atlantic Forest. Dark gray area represent the extent of Atlantic Forest Biome in Brazil. The circles of A1, A2 and A3 overlay the sampled sites distribution.

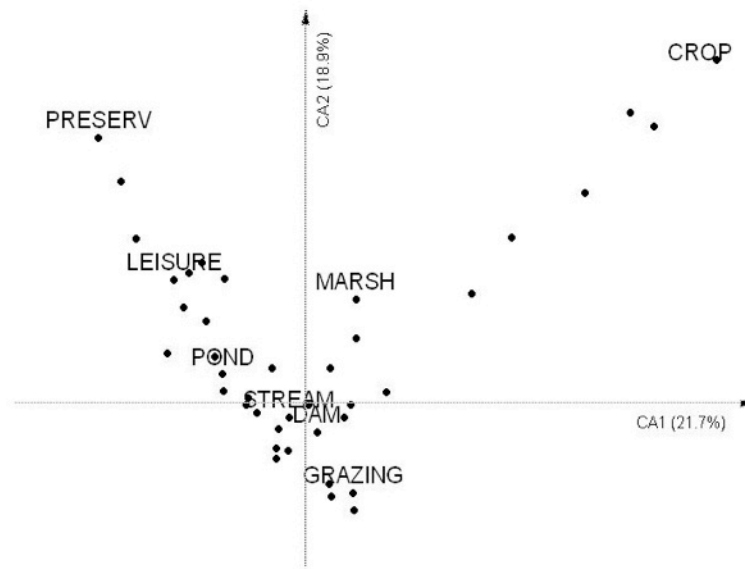


Figure 2. Scatterplot of the first two correspondence analysis axes (CA) of landscape descriptors for 90 sites. All descriptors scored as presence/absence. Percentages are the explained variation.

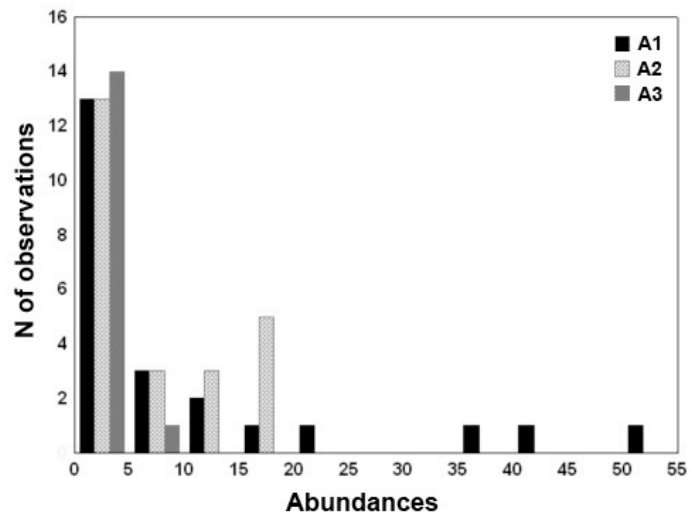


Figure 3. Histogram of *Lithobates catesbeianus* abundances observed in the three study areas.

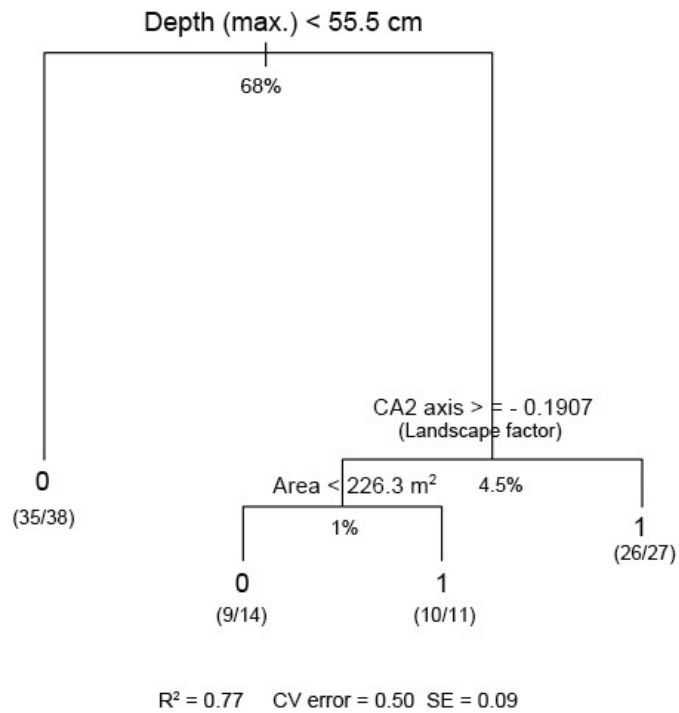


Figure 4 - Regression tree separating *Lithobates catesbeianus* breeding sites (1) from non-breeding sites (0). Percentage of explained variation shown below each node. Predicted values for each leaf and number of correct classifications relative to total number of observations (in parentheses) shown at leaves.

Appendix S1 - Geographic coordinates in decimal degrees of 90 waterbodies sampled in tree study areas of south Atlantic Forest in Brazil. The column “breeding” indicated sites where we found evidence of *Lithobates catesbeianus* breeding activities.

Area	Breeding	Latitude	Longitude	Area	Breeding	Latitude	Longitude
A1	1	-29.475083	-53.480000	A2	1	-26.869667	-52.899817
A1	0	-29.474383	-53.479650	A2	1	-26.879833	-52.900733
A1	1	-29.474150	-53.486400	A2	0	-26.880350	-52.904450
A1	0	-29.474500	-53.531717	A2	1	-26.886633	-52.921350
A1	0	-29.468433	-53.546533	A2	0	-26.890227	-52.924906
A1	1	-29.461722	-53.586133	A2	1	-26.881517	-52.925308
A1	0	-29.513367	-53.535817	A2	1	-27.139967	-52.752267
A1	1	-29.482417	-53.527300	A2	0	-27.139967	-52.752267
A1	0	-29.482367	-53.527283	A2	0	-27.155833	-52.751867
A1	1	-29.498517	-53.530467	A2	1	-27.163000	-52.751867
A1	1	-29.492033	-53.530467	A2	0	-27.165750	-52.753133
A1	0	-29.513483	-53.536067	A2	1	-27.144917	-52.664667
A1	1	-29.521100	-53.521950	A2	0	-27.144567	-52.660167
A1	1	-29.570567	-53.344383	A2	1	-27.143417	-52.652933
A1	0	-29.463850	-53.287333	A2	0	-27.142367	-52.652983
A1	1	-29.498833	-53.279817	A2	0	-27.144167	-52.653350
A1	1	-29.482067	-53.279150	A2	1	-27.141717	-52.655833
A1	0	-29.540983	-53.310800	A3	0	-26.706833	-49.106617
A1	1	-29.612167	-53.336350	A3	0	-26.693117	-49.112283
A1	1	-29.607517	-53.338600	A3	1	-26.713733	-49.092383
A1	0	-29.608967	-53.337933	A3	0	-26.714100	-49.092617
A1	0	-29.609667	-53.507183	A3	1	-26.714550	-49.092267
A1	0	-29.612133	-53.337183	A3	0	-26.730650	-49.052283
A1	0	-29.592700	-53.369167	A3	0	-26.945267	-49.270583
A1	0	-29.572167	-53.423350	A3	1	-26.931817	-49.255867
A1	1	-29.572950	-53.426750	A3	1	-26.946867	-49.266217

A1	0	-29.573467	-53.426683	A3	1	-26.946800	-49.265700
A1	1	-29.566400	-53.423433	A3	0	-26.947517	-49.265717
A1	1	-29.580000	-53.423100	A3	1	-26.947800	-49.265217
A1	0	-29.530483	-53.398817	A3	1	-27.003000	-49.119017
A1	0	-29.530467	-53.398383	A3	0	-27.005167	-49.118800
A1	1	-29.530683	-53.399333	A3	0	-27.005000	-49.118833
A2	1	-26.832067	-52.970167	A3	0	-27.009167	-49.119300
A2	0	-26.832117	-52.969450	A3	0	-27.014333	-49.119267
A2	1	-26.831650	-52.969250	A3	0	-27.005950	-49.119267
A2	0	-26.830633	-52.969783	A3	0	-27.006267	-49.111617
A2	1	-26.836350	-52.952550	A3	1	-27.036783	-49.111650
A2	1	-26.839400	-52.952450	A3	0	-27.037567	-49.112483
A2	0	-26.835500	-52.941450	A3	1	-27.037283	-49.112483
A2	0	-26.823700	-52.957283	A3	0	-27.037600	-49.111733
A2	0	-26.823683	-52.957133	A3	1	-27.030350	-49.093667
A2	1	-26.815383	-52.942600	A3	1	-27.053239	-49.086347
A2	0	-26.816200	-52.943100	A3	0	-27.056767	-49.088117
A2	1	-26.812867	-52.938317	A3	1	-27.053783	-49.086600
A2	1	-26.847500	-52.890083	A3	0	-27.057333	-49.086967

6. Capítulo III

**Amphibian richness in Atlantic Forest areas invaded by American bullfrogs:
may we expect low native richness where bullfrogs are abundant?**

**Amphibian richness in Atlantic Forest areas invaded by American bullfrogs:
may we expect low native richness where bullfrogs are abundant?**

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Abstract

The diversity–invasibility hypothesis is a central issue of biological invasion research. New theoretical and analytical approaches have revealed that spatial scale, land use factors and community assemblages are important predictors of the behavioral relationship between community diversity and invasibility. In this study we assess whether bullfrog abundance negatively predicts native amphibian species richness in Atlantic Forest waterbodies, and develop a model to understand how environmental, spatial and species composition gradients interplay within relationships between bullfrogs and native species richness. Once it was established that negative relationships between native species richness and invasive species were expected when invasions occurred in saturated communities, we tested for evidence of segregated spatial distribution in communities from invaded areas. We found only a weak relationship between bullfrog abundance and richness in invaded areas. The path model revealed that this relationship is indirect, mediated by community composition gradients. Our results indicated that bullfrogs are more abundant in some specific amphibian compositions, which may be species rich. Local filters describing habitat complexity were the main predictors of species richness, composition and bullfrog abundance. We did not find any evidence of saturation in invaded communities, or of bullfrogs causing changes in species co-occurrences patterns. Our results reinforce the habitat roles for species diversity and invasibility.

Keywords: *Lithobates catesbeianus*, anurans, diversity, invasion, Brazil.

Introduction

The migration of novel species into communities or uncolonized patches is an important process for community development, structure and composition (MacArthur and Wilson 1967; Connell and Slatyer 1977; Loreau and Mouquet 1999). We are currently witnessing an age of biological invasions, with novel species been incorporated into local communities not only by dispersal, but by intentional or accidental introductions. Such invasions are not restricted to taxa, regions, biomes or continents (Soulé 1990) and have drawn attention because they may become economically expensive. For instance, exotic species may negatively affect crops, fisheries, public health systems and so on, meaning the loss of billions of dollars (Pimentel et al. 2001). Nevertheless, invasions could be also expensive in an ecological and evolutionary context, since they are linked to recent species extinctions (Clavero and Garcia-Barthou 2005).

Charles Elton prophesied the present scenario (Simberloff 2011), and since his seminal synthesis in “The ecology of invasions by animals and plants” (Elton 1958) interest has been growing in identification of those species traits that predict invasion risk, and community features facilitating or preventing invasions. In his book, Elton observes that: i) “populations in simpler communities are more prone to population fluctuation”; and ii) “tropical systems are less invaded than species poor temperate and boreal systems”. Both statements are linked to the roots of the diversity–invasibility hypothesis (see Justus 2008). These statements convey the idea that diverse systems should fluctuate less, and thus be more stable. Elton associates the “tropical resistance” with highly diverse communities, sheltering large numbers of enemies and parasites, which in their turn may impede the establishment of novel species. Elton himself recognized that this should only partially explain the low invasion rates in the Tropics,

and future studies explained the diversity–invasibility hypothesis in the light of resource exploration: more rich communities tend to be saturated and to explore resources in complementary ways (MacArthur and Levins 1967; MacArthur 1970).

The diversity–invasibility hypothesis is still a central issue in ecology, and the related empirical evidence includes some inconsistent results (Fridley et al. 2007). There is evidence from observational studies to show that alien species diversity is low in richer systems, as although some studies found no relationship at all (e.g. Case and Bolger 1991). Within experimental studies, there is evidence both supporting a negative relationship between diversity and invasion (e.g. Tilman 1997), and also a positive relationship (e.g. Robinson et al. 1995), and even studies highlighting a lack of relationship (e.g. Robinson and Dickerson 1984).

The presence of such contrasting results has been called “the invasion paradox” (Fridley et al. 2007), and seems to be related to a spatial scale issue. In an extensive review, Fridley et al. (2007) demonstrated that while in large scale studies the relationship between invasive species and native richness seems to be positive, at smaller scales, especially in experimental studies, negative relationships are often found. Nonetheless, it is known that a positive relationship between non-native and native species could be found only by virtue of a sampling artifact (Fridley et al. 2004). Apparently positive or negative relationships may also be a result of analytical models ignoring important co-variables, which may explain both invasive species abundance or richness and native species diversity (Taylor and Irwin 2004). Factors such as propagule pressure, disturbances and others environmental gradients often work together to explain invasion patterns (Elton 1958; Von Holle and Simberloff 2005). Therefore in situ studies, considering distinct spatial scales and addressing the native richness and invasive species

relationship, are still required for proper understanding of the diversity–invasibility hypothesis, especially with regard to approaches considering causal links connecting environmental and community gradients.

In many systems, invasions are concrete reality, despite several decades of research and efforts to its prevention, and they will become even more conspicuous (Richardson 2011). In already invaded ecosystems, rather than to understand if diversity will prevent invasions, we are challenged to estimate the invasion effects upon diversity. The problem relies in the fact that once invasion attain fully invasive stages, without pre-invasion data, we would not be able to say if invasive species already promoted species loss in the past. Even so, we may look at current invasion effects upon community diversity patterns, asking whether invasion predict poorer communities, or composition changes, while controlling for environmental and spatial related variation.

Classical examples of invasion effects in ecosystem diversity come from freshwater habitats. They are among the most endangered ecosystems and have historically suffered from structural modifications, even though biotic modification imposed by the addition of invasive species is the main cause of extinction of freshwater species (Witte et al. 1992; Doods and Whiles 2010). Waterbodies such as lakes, ponds, pools, and temporary streams can be viewed as small islands, a system which seems to be very sensitive to invasions (Ricciardi and MacIsaac 2011). Non-native fishes are distributed across the globe (Lever 1996) and constitute the best historical example of major impacts upon native communities by trophic cascade effects in freshwaters (Moyle and Light 1996). The American bullfrog (or bullfrog), *Lithobates catesbeianus* (Shaw 1802) is one of the freshwater invasive species integrated into the “hall of fame”. It is cited on the list of the 100 worst aliens of the world (Lever 2003), because it is one

of the causes of amphibian decline in North America and appears to be an important vector of fatal amphibian diseases, such as the chytridiomycosis (Daszak et al. 2004; Schloegel et al. 2010). The species is benefited by human-modified landscapes (Adams 1999; Zampella and Bunell 2000; D'Amore et al. 2010; Fuller et al. 2011), non-native fish presence in freshwaters (Adams & Wasson 2000), and from simple aquaculture enclosures that ensure a continuous source of propagules (Liu and Li 2009).

Bullfrogs originate from eastern North America, and are distributed from Mexico to south Canada, but invasive populations occur in western North America, Europe, Asia, and central and southern American countries, where they were introduced for aquaculture practices (Santos-Barreira et al. 2009). In invaded sites, they could be negatively correlated with native frog abundance, and with occurrence of specific native frog species; they may also reduce tadpole survival (Kupferberg 1997; Kiesecker and Blaustein 1998; Kats and Ferrer 2003; Wang and Li 2009), and are often implicated as a factor in the decline of native amphibians and potentially in species loss (Kraus 2009). A negative relationship between bullfrogs and native richness is reported from China (Li et al. 2011). Bullfrogs have been present in Brazil since the 1930s and invasions have continued to occur since then, probably growing exponentially from the 1970s to 1990s when bullfrog farms received incentives (Lima and Agostinho 1988). It is known that Brazil is very suitable for bullfrog establishment; the Atlantic Forest is especially suitable, and climate change may make forest protected areas even more prone to invasion by bullfrog populations (Nori et al. 2011; Loyola et al. 2012). We do not know what is the pattern of relationships between bullfrogs and diversity of native communities; however we do know that they are currently widespread in Brazil (Both et al. 2011b), are already present in protected areas and at a fully invasive degree (*sensu* Blackburn et al. 2011), at least in the south Atlantic Forest (Both et al. 2012).

In this study, we tested relationships between the abundance of a non-native species and native diversity in areas where the invasion had reached a fully invasive degree, and we were not therefore seeking to determine if diversity can prevent invasions. Our primary goal in this study was assess how amphibian Atlantic forest communities vary in richness in invaded areas and whether bullfrog abundance negatively predict richness patterns. We expected such a negative relationship because we were investigating richness and invasibility relationship at a fine spatial scale. Furthermore, we developed a model to predict how environmental, spatial and species composition gradients interplay in the relationships between bullfrogs and native species richness. Amphibian species replace along waterbody gradients (Wellborn et al. 1996; Van Buskirk 2005; Both et al. 2011a), whereas bullfrogs are highly aquatic, linked with permanent and deep waterbodies (Bury and Whelan 1984; Adams 2003); we therefore expected that species using other resources should be either less or not affected by bullfrog populations.

Considering that negative relationships between native richness and invasive species are expected when invasions occur in saturated communities (Elton 1958; MacArthur 1970), but also according to spurious correlation at small scales (Fridley et al. 2004), we tested for evidence of segregated spatial distribution in communities from invaded areas. As we did not have community data dating previous to the invasion, our analysis did not cover the effects of bullfrogs on communities, but instead only indicated if saturation was potentially occurring.

Materials and Methods

Study Areas

The study was conducted in three different areas of the Atlantic Forest domain in southern Brazil: the central region of Rio Grande do Sul State, and the west and east respectively of Santa Catarina State. These areas are high susceptible for bullfrog invasive populations and are within regions in which are concentrated the known occurrence records of *L. catesbeianus* in Brazil (Both et al. 2011b). Invasions date from a minimum of 10 years ago in all cases. Rio Grande do Sul (study area A1), comprises Agudo, Dona Francisca, Ivorá and Nova Palma Municipalities. In this area the vegetation is characterized by Seasonal Deciduous Forest (IBGE 2004). In the west of Santa Catarina (study area A2) are included Chapecó, Guatambu, Nova Erechim and Pinhalzinho municipalities. In this area of the Atlantic forest two phytogeographic forms occur, including Seasonal Deciduous Forest and Mixed Ombrophilous Forest (IBGE 2004; Lucas and Fortes 2008). In the east of Santa Catarina (study area A3) are included the municipalities of Blumenau and Indaial; the area is vegetated by Dense Ombrophilous Forest (IBGE 2004). The Atlantic Forest is highly fragmented in the three study areas. The general land use is farming and cattle grazing. A more detailed description, including geographic coordinates, is provided in Both et al. (2012).

Sampling

We sampled a total of 90 waterbodies: 32 in A1, 30 in A2 and 28 in A3. In each area half of the waterbodies were breeding sites of *Lithobates catesbeianus*, and half were sites not used for breeding, spatially alternated. They were selected in a pilot survey conducted in spring 2009. There were no active bullfrog farms near the study areas. Postmetamorphic individuals and tadpoles were sampled twice at all sites, in February/March (late summer) and October/November (spring) of 2010. Sampling occurred at

periods of longer photoperiod, which is a predictor of high amphibian breeding activity periods in subtropical regions (Canavero and Arim 2009).

On each day of sampling, we sampled four to six sites for eggs and tadpoles in the daytime, and postmetamorphic individuals at night. We sampled tadpoles in distinct microhabitats (e.g., marginal and without vegetation, centre with and without vegetation) with dip net sweeps (40 x 30 cm, 0.02 mm mesh), always using the same collector. The effort was proportional to pond size and heterogeneity (Shaffer et al. 1994). A minimum of five sweeps were undertaken in each microhabitat. Samples were taken between 09:00 and 19:00. Postmetamorphic surveys were begun 30 minutes after sunset, and we searched for individuals along the perimeters of breeding sites. We counted all postmetamorphic individuals, accounting for separation of calling and non-calling individuals. We employed effort proportional to waterbody size and complexity (Scott and Woodward 1994).

Environmental descriptors

We accounted for the presence of other waterbodies within a 400 m radius for each site, considering marshes, ponds, streams and dams. Within the same radius, we also classified the surrounding land use as grazing (cattle, sheep), crops, preservation (reserves), and leisure (private properties used as weekend holiday homes, without economical purpose). All these data were converted into binary descriptors. Here, we used landscape factors obtained by combining the data set through correspondence analysis (CA), as described in Both et al. (2012). The first three coordinates of CA analysis explained 57% of the variation, and were considered to be landscape factors. The first axis reflected the order of preserved–grazing–crop sites, respectively. The

second axis reflected waterbody availability. Descriptor scores for the first three CA axes are provided in Table 1. We measured the distance to the next forest fragment, and the distance to the next road, using GPS. We expanded these distances in second and third order monomials to test for potential nonlinear relationships with richness.

Area and depth of each waterbody was measured at each collection event. For depth, we took the measurements in each of the microhabitats sampled for tadpoles and recorded the mean depth, and the maximal and minimal values from all microhabitats for each site. The ratio between the areas of the two sampling events (summer area/spring area) was used as a measure of pond permanence. We recorded richness of hydrophyte morphotypes (floating and emergent macrophytes) in the pond. We visually estimated the hydrophyte coverage and classified it into three classes: < 30%, 30–60% and > 60%. Fish presence was determined through visual surveys, dip net captures or was based on information provided by local rural owners. We classified the vegetation of waterbody banks into grassy low, grassy high, shrubs and arboreal, and used the sum of types present in a site as a descriptor of the bank vegetation structure.

Spatial models

The spatial structure of sites in this study had two major scales: between areas (A1, A2 and A3) and within areas. To account for this nested spatial arrangement, we considered two kinds of spatial descriptors in the matrix S: the three large study areas, represented by dummy variables, and Moran's eigenvector maps (MEM). The seven MEMs used in this study were the same as described in Both et al. (2012). They were built using a nested MEM model as proposed in Declerk et al. (2011), in which MEM variables are blocked within study areas, describing spatial relationships at this scale. These MEM

models were built using the matrix of dummy variables describing the three regions as the connectivity criteria, and distances of sites within areas.

Data analyses

Richness and composition

To remove the effects of sampling effort on richness values, we regressed richness on abundance of postmetamorphic individuals, which showed a 96% concordance with the total richness, and used the residuals, henceforth called richness, in subsequent analyses.

To describe species composition across waterbodies, we calculated Jaccard's index of similarity for communities pairs and performed a Principal Coordinate Analysis (PCoA) (Legendre and Legendre 1998), using only presence–absence data. We chose occurrence data instead of species abundances because it allowed us to combine postmetamorphic and tadpole surveys, thus dispensing further weighting assumptions upon the importance of distinct life history stages. The stability of ordination axes was evaluated by bootstrap resampling (Pillar 1999). The PCoA analysis was performed in Multiv (Pillar 2006).

Firstly, we regressed richness on bullfrog abundance to test for the existence of a relationship. Secondly, we tested whether species richness is related to environmental and spatial descriptors, jointly with community composition and bullfrog abundance, through path analysis. This analytical approach allowed us to depict the covariation structure between predictors, and potentially to reveal novel causal links (Shipley 2000). We started building a hierarchical theoretical model of potential causal relationships explaining native species richness (Figure 1). We tested this model using the analytical steps proposed by Brum et al. (2012): i) we performed separated regressions between native richness and each set of descriptors to select which would of these would be used

in the model; ii) we then regressed all the selected variables with richness to test for direct causal links; iii) obeying the causal hierarchy, we regressed all variables directly linked with richness with their respective potential predictors, and so on, until we reached the exogenous descriptors, or spatial descriptors in this case. For instance, if a waterbody descriptor was directly related to local richness, we further investigated which landscape and spatial descriptors could explain this descriptor. If a landscape descriptor was selected, we inspected its relationships with high order spatial descriptors. Fish presence was considered together with other waterbody descriptors; however we investigated further causal links associated with this variable (see Figure 1), because it is a well-known filter for lentic communities (Wellborn et al. 2006). β regression coefficients were taken as path coefficients and we used the AIC criterion for all model selections (Anderson 2008). All regression models were performed in R 2.5.1 (R Development Core Team, 2012).

Community assembly patterns in invaded areas

We compiled a presence–absence matrix for each study area, where each row was a species and each column was a waterbody. We used the C-score index of co-occurrence (Stone and Roberts 1990) to test for community structure. The C-score measures the average number of checkerboard units (CU) between all species pairs. In a saturated community, we would expect to find a high C-score value, indicating low co-occurrence of species pairs. We compared the observed index for each with an index obtained for 10,000 random communities.

Null models, including all species and bullfrogs occurrences, were constructed following a fixed–fixed algorithm. The fixed–fixed algorithm built null communities

where sites always receive the same number of species of the observed matrix and species always occur in the observed frequency. Therefore richness differences between sites and species frequency of occurrence are respected

The null models comparing co-occurrences on sites where bullfrogs occur with sites where they are absent were constructed using partition tests as null models (Stone et al. 2000). For this model, we removed bullfrog occurrences from presence–absence matrices, because bullfrog presence was used to distinguish sites as “invaded” or “non-invaded”. The C-score for these two types of site was then compared with the score expected for one of 5000 random replacements for “invaded” and “non-invaded” sites. We then observed the variance of these null communities. Larger variances indicated that co-occurrence patterns were distinctive between the two types of sites. All co-occurrence analyses were carried out using EcoSim (Gotelli and Entsminger 2007).

Results

Across the three studied areas we recorded 40 native amphibian species: 19 species in A1, 19 in A2 and 23 in A3 (Table II). Native amphibian richness ranged from zero to 11 species, with an average of 3.9 species per waterbody (SD = 2.8). The species composition ordination resulted in 37 PCoA axes. The first three axes represented 41% of species composition gradients and were used in the regression models. The stability of the first three ordination axes was validated by bootstrap resampling evaluation. The first two axes represented the composition gradient from ombrophilous, changing to mixed ombrophilous composition, to seasonal deciduous forest amphibian communities Figure 2A. The first principal coordinate (PC1) axis clearly varied from amphibian compositions restricted to dense ombrophilous forest, e.g. *Sphaenorhynchus* sp. and

Bokermanohyla hylax, from compositions of species occurring at high frequency across study regions, such as *Dendropsophus minutus* and *Physalaemus cuvieri* (Figure 2B).

The linear model relating native amphibian richness with bullfrog abundance showed that they are weakly positive related (Table III). Richness was also positively related with the first two principal coordinates of sites composition. Three MEMs were selected in the spatial model: MEM.2, which described spatial structure within A2; MEM.3, which described spatial structure within A3; and MEM.7 describing spatial structure within A1. The landscape model was not validated. The model relating richness with local waterbody descriptors selected maximal depth, hydrophyte richness, bank vegetation structure, fish presence and streams as predictors (Table III).

The path model showed that native amphibian richness was directly determined by the first two principal coordinates, PC1 and PC2, maximal depth and the binary descriptors fish presence, as well as by one spatial model, MEM.2 (Figure 3). The composition gradients showed the highest path coefficients. Stream waterbodies and two spatial models, MEM.3 and MEM.4, did not show significant causal link with amphibian richness. Bullfrog abundance only indirectly predicted species richness through its relationship with the community composition gradient PC1. Hydrophyte richness were also only indirectly linked with richness through the composition gradients. It also directly determined bullfrog abundance and community composition gradients PC1 and PC2 (Figure 3). The final model accounted for 63% of native richness variation.

The co-occurrence analysis did not indicate that species are segregated in any of the three study areas (Table IV). The assemblages from ombrophilous forest showed only a marginal tendency towards spatial segregation. When co-occurrence patterns between waterbodies supporting *Lithobates catesbeianus* breeding populations were

compared with the remaining sites, the co-occurrence pattern also did not differ from that expected by random for the three regions (Table IV).

Discussion

Diversity–invasibility research has been changing over time, concomitantly with new and/or mass invasions, new theoretical approaches, and with the increase of available analytical tools. To separate the respective roles of space, environment, local communities and human activities upon invasion patterns and respective resultant processes is a difficult task. Changes in habitat may promote invasion increase, or the increase of one non-native species abundance. The latter may apparently influence species decline, but could be only a proxy variable of ecosystem degradation (Didham et al. 2007). Such incorporation of alternative hypotheses and refined models has been promoting advances in the biological invasion research (Sax et al. 2007). While we continue to expand our view of the process of biological invasions, we will keep changing or improving the main related mechanistic hypotheses. Currently it is an urgent need to understand how invasions affect community diversity.

In this study, we were expecting a negative relationship between bullfrog abundance and native amphibian richness, because we were considering diversity patterns at a local scale and in fully invaded areas. Contrary to our initial expectations, we found only a weak and positive relationship, which was later revealed to be an indirect path link explained by composition gradients. Local environmental gradients directly explained all biotic components of the model, also showing an additional and important influence upon richness through species composition gradients. Bullfrog abundance also responded to the same gradients. Across the three Atlantic forest areas, space showed

only a low influence upon richness patterns, and we found only weak relationships of micro-regions with lower richness, in A1 and A2, and with higher richness, in A3, but one spatial model was causally significant when all covariation structure was considered.

Here, we tested if the bullfrog abundance as a factor predicting richness, and observed that its relationship with richness occurs through composition gradients. We can say that bullfrogs tend to be more abundant in certain communities, and that these communities may be species rich. Bullfrogs positively predicted the PC1 community gradient, varying in a similar fashion with very common species such as *Physalaemus cuvieri* and *Dendropsophus minutus*, which show broad range distributions in South America (IUCN 2011). These species are cited as anthropogenic adapted (Santos et al. 2007), similar to bullfrogs. These results are similar to those of Bunnell and Zampella (2008), who found that bullfrogs are associated with distinct compositions, and more closely related with species that have a widespread distribution. However, bullfrogs do co-occur with other community compositions, but at lower abundances, and may be affecting communities by other pathways.

Our results highlighted how all biotic components, species richness, composition and bullfrog abundance, respond to local filters directly or indirectly. Hydrophyte richness and waterbody maximal depth were the main predictors selected in our final model. These predictors could be viewed as a measure of habitat complexity, which undoubtedly plays an important role for both faunal biodiversity and abundance, through its potential to decouple trophic interactions and, in doing so, to promote diversity (Kovalenco et al. 2012). We assessed only a simplistic measure of structure on the water surface, richness of hydrophyte morphotypes, and found that it was still a important predictor of community gradients. Hydrophytes could promote diversity by providing

shelter for tadpoles and postmetamorphic individuals, and by working as calling sites or for postmetamorphic. Maximal depth is a gradient that contains variation related to pond area, permanence, availability of other lower depths, and is also a very important resource for communities in small waterbodies. It governs the coexistence of nektonic and benthic tadpoles in ponds and streams (Eterovick and Fernandes 2001; Both et al. 2011c). Fish presence and bullfrog presence are also regulated by this gradient, because only waterbodies with a certain depth may support these organisms, and as tadpoles, fishes replaces across depth gradients (Kushlan 1976).

The role of habitat complexity for a native frog population in invaded areas in North America was highlighted by Adams et al. (2011). They did not find supporting evidence for an effect of bullfrogs on the local extinction probability for the native species, and showed that vegetation cover and riparian vegetation are the best predictors of low extinction risk at local scales for a native frog. The relationships between species composition and bullfrogs with local filters are encouraging, because they open the opportunity of habitat management to protect native frogs (Both et al. 2012). We observed that depth was causally linked with bullfrog abundance and species richness through positive and negative paths. Further evidences come from studies evaluating the importance of disturbed hydrological regimes, which showed that waterbody modification favor bullfrogs and promote composition changes (e.g. Bunnell and Zampela 2008; Fuller et al. 2011). In south Atlantic forest areas, the best predictor for bullfrog breeding population presence and abundance is depth, and it is known that a major contribution to maintenance of deep waterbodies is provided by agricultural dams (Both et al. 2012).

The co-occurrence models in this paper found no evidence of saturated communities, nor any evidence of distinct co-occurrence patterns in sites supporting bullfrog breeding populations. Segregation between guilds, time or season may still occur, but bullfrogs are probably not influencing these patterns. It is worth noting that only recent studies dealing with bullfrog invasion patterns highlight neutral relationships between bullfrogs and richness, or emphasize the role of habitat filtering to promote or facilitate invasions (e.g. D'Amore 2010; Adams et al. 2011). On the other hand, it is also interesting that other regions where bullfrogs have established invasive populations have communities sharing a more recent phylogenetic relatedness with bullfrogs. In Asia, Europe, and especially North America there are more Ranid species. For instance, in China where a negative relationship with richness is known to occur, native species composition is mostly of ranids (Li et al. 2011). The potential influence of relatedness upon invasions was recognized by Darwin (1859) in "The Origin of Species." Darwin suspected that low degree of relatedness should give some advantage to novel species in new habitats. Novel species may occupy empty niches (see Tilman 2004). It may be that the novelty is not a relevant factor in South America invasion, but this theme deserves further study considering the global scale invasion of bullfrogs, and many other alien species.

In conclusion, we found only a weak and indirect relationship between native frog richness and bullfrog abundance mediated by composition gradients in Atlantic Forest invaded areas, in the context of the main conclusions of recent studies which test alternative hypotheses and consider the related covariation of environmental or land use descriptors with invasion patterns. Our results indicated that bullfrogs are more abundant in some amphibian compositions, which may be species rich. The main result is that local filters are predicting native richness, composition and bullfrog abundance.

Furthermore, we are not concluding any absence of effects upon native species at present. Disease transmission, differential predation and competition by interference are mechanisms which could plausibly be operating in invaded areas even when bullfrogs show low abundances, and deserve future attention.

Acknowledgments

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Table 1 - Landscape descriptor coordinates for the first three ordination axis in correspondence analysis.

	CA1	CA2	CA3
Dam	0.08	-0.19	0.40
Stream	-0.12	-0.09	-0.14
Marsh	0.34	0.62	0.44
Pond	-0.57	0.17	-1.01
Grazing	0.33	-0.56	-0.02
Crop	2.81	2.07	-0.59
Leisure	-0.90	0.74	1.36
Preservation	-1.42	1.61	-0.23

Table 2 - Amphibian composition from southern Atlantic Forest areas invaded by *Lithobates catesbeinaus*. A1 = central region of Rio Grande do Sul, deciduous forest; A2 = western of Santa Catarina, deciduous and mixed ombrophilous forest; A3 = east of Santa Catarina, ombrophilous forest.

Species	Abbreviation	Postmetamorphics	Tadpoles	A1	A2	A3
Bufonidae						
<i>Rhinella abei</i>	Rab	1	1			1
<i>Rhinella fernandezae</i>	Rfe	1		1		
<i>Rhinella icterica</i>	Ric	1		1	1	1
Cycloramphidae						
<i>Limnomedusa macroglossa</i>	Lma	1		1		
Hylidae						
<i>Bokermannohyla hylax</i>	Bhy	1	1			1
<i>Dendropsophus minutus</i>	Dmin	1	1	1	1	
<i>Dendropsophus microps</i>	Dmic	1	1			1
<i>Dendropsophus nahdereri</i>	Dnah	1				1
<i>Dendropsophus nanus</i>	Dnan	1	1	1		
<i>Dendropsophus sanborni</i>	Dsa	1	1	1		
<i>Dendropsophus werneri</i>	Dwe	1	1			1
<i>Hypsiboas albomarginatus</i>	Halb	1	1			1
<i>Hypsiboas albopunctatus</i>	Halbp		1		1	
<i>Hypsiboas bishoffi</i>	Hbi		1			1
<i>Hypsiboas faber</i>	Hfa	1	1	1	1	1
<i>Hypsiboas pulchellus</i>	Hpu	1	1	1		
<i>Hypsiboas semilineatus</i>	Hse	1	1			1
<i>Hypsiboas</i> cf. <i>semiguttatus</i> 1	Hsm1		1		1	
<i>Hypsiboas</i> cf. <i>semiguttatus</i> 2	Hsm2		1	1		
<i>Phyllomedusa tetraploidea</i>	Pte	1	1		1	
<i>Phyllomedusa distincta</i>	Pdi	1				1
<i>Scinax alter</i>	Sal	1				1
<i>Scinax fuscovarius</i>	Sfu	1	1	1	1	1
<i>Scinax granulatus</i>	Sgr	1	1	1	1	1
<i>Scinax perereca</i>	Spe	1	1		1	1
<i>Sphaenorhynchus</i> sp.	Sphe	1				1
Hylodidae						
<i>Crossodactylus</i> sp.	Cro		1		1	
Leiuperidae						
<i>Physalaemus gracilis</i>	Pgr	1		1	1	1
<i>Physalaemus cuvieri</i>	Pcu	1	1	1	1	1

<i>Physalaemus nanus</i>	Pna	1	1			1
<i>Pseudopaludicola falcipes</i>	Pfa	1	1	1	1	
Leptodactylidae						
<i>Leptodactylus fuscus</i>	Lfu	1	1	1	1	1
<i>Leptodactylus gracilis</i>	Lgr	1	1	1		1
<i>Leptodactylus labyrinthicus</i>	Lla		1		1	
<i>Leptodactylus joly</i>	Ljo	1				1
<i>Leptodactylus latinasus</i>	Lla	1	1	1		
<i>Leptodactylus latrans</i>	Llat	1	1	1	1	1
<i>Leptodactylus mystacinus</i>	Lmy	1	1			1
<i>Leptodactylus plaumani</i>	Lpl	1			1	
Microhylidae						
<i>Elachistocleis bicolor</i>	Ebi	1	1	1	1	
Total		34	30	19	19	23

Table 3 - Linear model relating native amphibian richness and bullfrog abundance and selected models relating richness with community gradients, waterbody descriptors and spatial models. Model selection was based on corrected Akaike information criterion.

	R ²	AICc	AICc wi
Bullfrog Abundance	0.05	405.17	-
Community gradient PC1 + PC2	0.51	348.24	0.75
Waterbody descriptors Depth _{max} + bank vegetation structure + hydrophyte richness + stream + fish presence	0.31	384.94	0.27
Spatial descriptors MEM.2 + MEM.3 + MEM.7	0.14	401.22	0.01

Table 4 - Values of observed C-score and mean of C-scores obtained in 10,000 null simulations. Models of species co-occurrence built using fixed-fixed algorithm.

Species co-occurrences			
Area	C-score _{obs}	C-score _{sim}	P _{obs>sim}
A1	8.837	8.923	0.578
A2	13.310	13.285	0.458
A3	9.740	9.440	0.083
Breeding and non-breeding sites structure			
	Bullfrog breeding sites C-score variance	Non-breeding sites C-score variance	P _{obs>sim}
A1	0.371	0.360	0.604
A2	0.970	0.715	0.321
A3	0.218	0.334	0.753

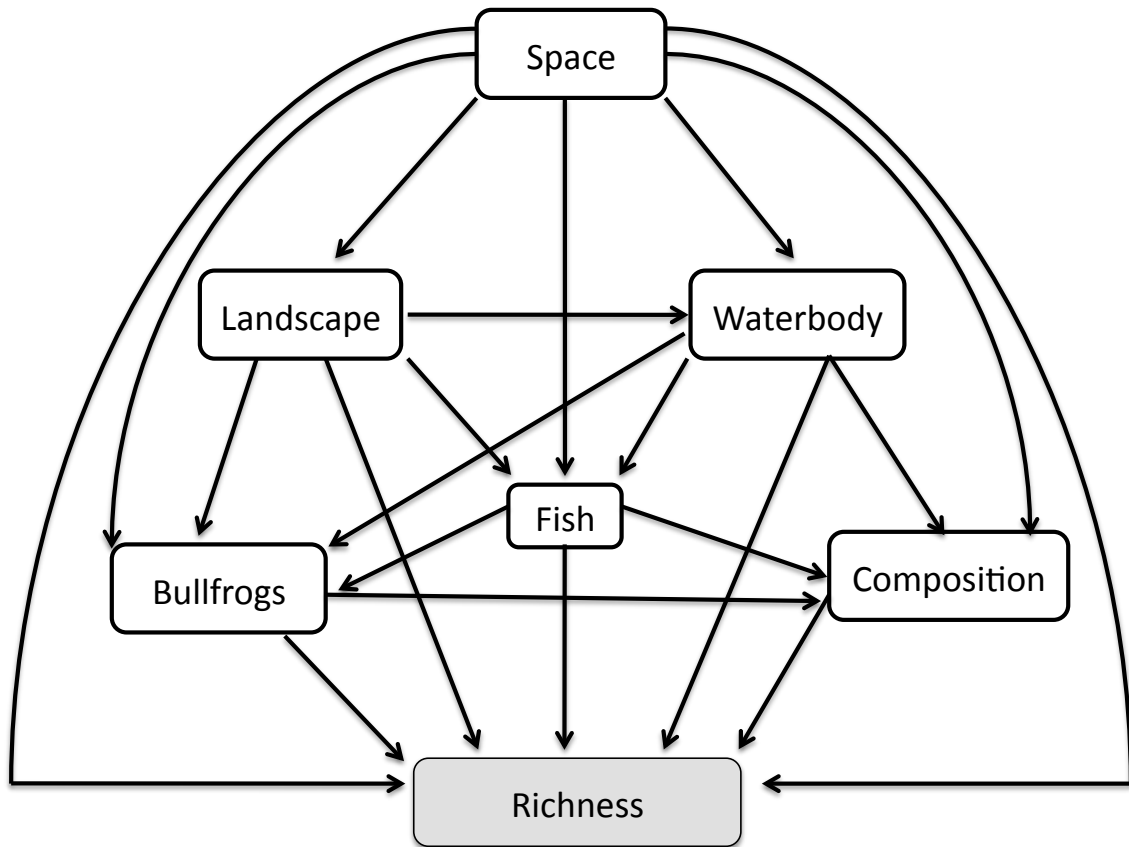


Figure 1 - Theoretical path model explaining relationships of native amphibian richness with community gradients, space, landscape, waterbody descriptors and American bullfrogs abundance in invaded areas.

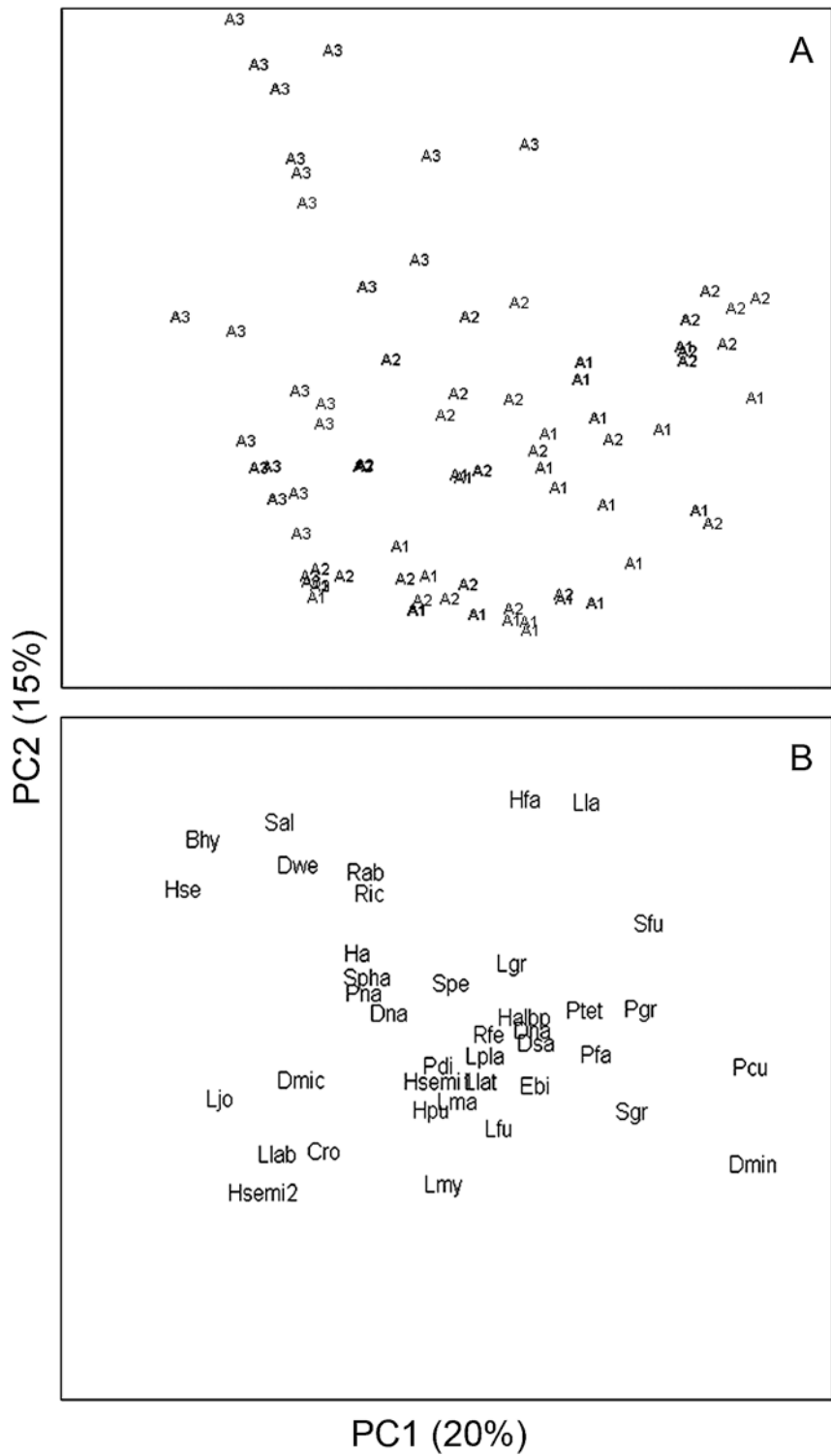


Figure 2 - Ordination of amphibian species composition across three study areas in A. Euclidian representation of species correlation with the first two ordination axes in B. Legend for species abbreviations is provided in Table 2.

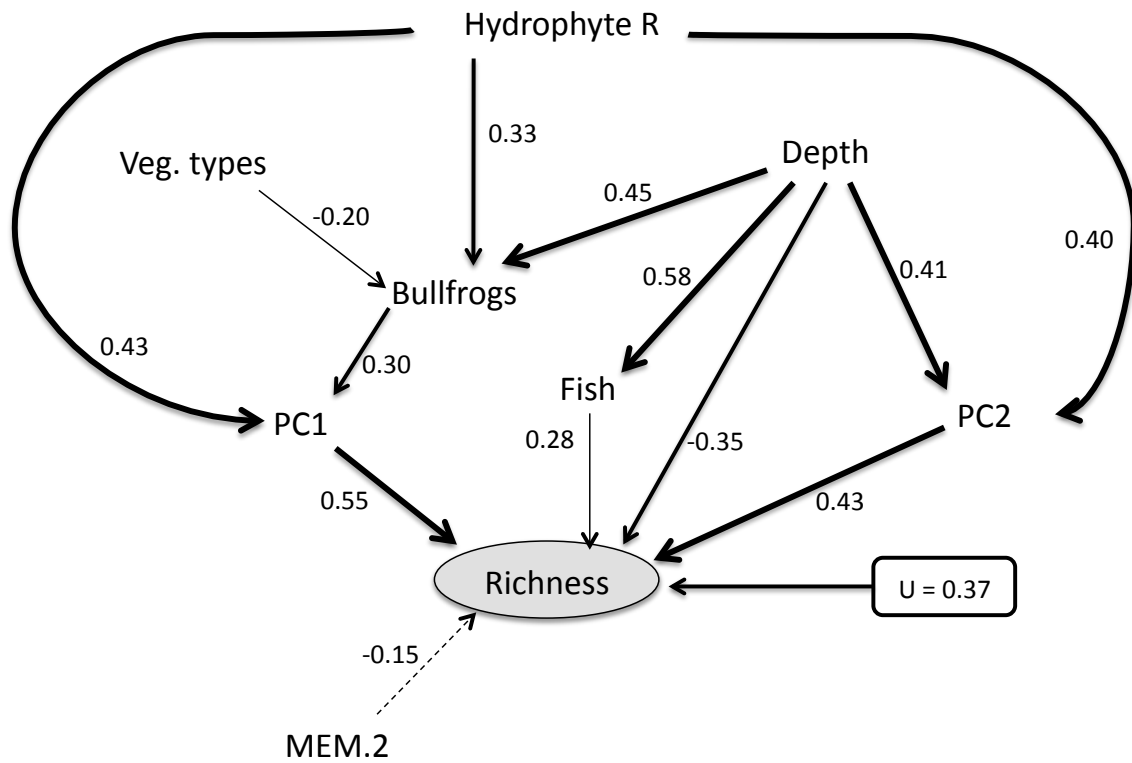


Figure 3 - Causal relationships between selected spatial and environmental predictors, amphibian species composition gradients, bullfrogs abundance and native amphibian richness. U is the non-determination coefficient ($U = 1 - R_2$). Only significant paths are represented.

7. Capítulo IV

Biological invasions and the acoustic niche: the effect of bullfrog calls on the acoustic signals of white-banded tree frogs

**Biological invasions and the acoustic niche: the effect of bullfrog calls on the
acoustic signals of white-banded tree frogs**

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Abstract

Invasive species are known to affect native species in a variety of ways, but the effect of acoustic invaders has not been examined previously. We simulated an invasion of the acoustic niche by exposing calling native male white-banded tree frogs (*Hypsiboas albomarginatus*) to recorded invasive American bullfrog (*Lithobates catesbeianus*) calls. In response, tree frogs immediately shifted calls to significantly higher frequencies. In the post-stimulus period, they continued to use higher frequencies while also decreasing signal duration. Acoustic signals are the primary basis of mate selection in many anurans, suggesting that such changes could negatively affect the reproductive success of native species. The effect of bullfrog vocalizations on acoustic communities are expected to be especially severe due to their broad frequency band, which masks the calls of multiple species simultaneously.

Keywords: invasion, acoustics, noise pollution, bullfrog, *Hypsiboas albomarginatus*, Anura, Amphibia

1. Introduction

Biological invasions contribute to biodiversity loss, ecosystem degradation, and impairment of ecosystem services worldwide [1]. Indeed, invasive species are associated with over 50% of the recent extinctions of animal species for which causes can be compiled [2]. They also provide invaluable insights into ecosystem functioning and evolutionary processes that complement and inform broader scale studies in systematics and biogeography [3].

Interactions between native and invasive species have been studied from a variety of perspectives; however, the potential consequences of invasion of the acoustic niche have not been explored: how do native species respond when a new acoustic competitor appears? Many animal species employ acoustic signals to attract and assess potential mates and evaluate rivals. Environmental sounds can impede acoustic communication by attenuating and degrading signals and reducing signal-to-noise ratio [4]. In response, acoustic species can rapidly modulate signal parameters, including rate, timing, amplitude, and frequency, which can result in altered female mate preference and decreased reproductive success [5,6]. In addition to the environmental noises that have been studied previously [7], sounds produced by invasive species might also cause native species to modulate acoustic signals.

To study the effects of an acoustic invader on native callers, we simulated an acoustic invasion by exposing calling native male white-banded tree frogs (*Hypsiboas albomarginatus*) to invasive American bullfrog (*Lithobates catesbeianus*) advertisement calls. Based on previous studies of the effect of environmental noise on anurans [8–10], we predicted immediate changes in signal rate, and we also tested for changes in other gross-temporal calling patterns and spectral properties.

2. Materials and Methods

Study area and focal species

The study was conducted from November to December 2009 at three permanent ponds in an Atlantic Forest relict in Serra do Itajaí National Park, Blumenau, Brazil (27°03'S, 49°05'W). Pond areas were 306–16,310 m². The park road was 500 m from the closest pond and we did not record any anthropogenic sounds during the experiments.

White-banded tree frogs (*Hypsiboas albomarginatus*) occur in the Atlantic Forest and breed in ponds near forest edges. Breeding males emit advertisement calls of one or two multi-pulsed notes with harmonics at 1060–1430 Hz and 2720–2780 Hz or 2280–2850 Hz [11]. American bullfrogs (*Lithobates catesbeianus*) are native to eastern North America but are currently widespread in the Atlantic Forest [12]. During the study period, bullfrogs were active in the region and we observed calling males, eggs, tadpoles, and non-calling individuals in similar ponds also inhabited by *H. albomarginatus*. However, in four field expeditions in 2009 and 2010 we failed to detect tadpoles or post-metamorphic bullfrogs at or near any of the study ponds. Bullfrog advertisement calls cover a broad frequency band (90 to > 4000 Hz) with energy peaks at 200–400 Hz and 1000–2000 Hz [13].

Playback experiment

To assess calling patterns of invasive individuals in southern Brazil, we recorded 5 min of continuous calling by 10 solitary bullfrogs at Novo Treviso, Faxinal do Soturno municipality (29°34'S, 53°26'W). Solitary males emitted 3–7 consecutive calls with intervals of 15 s to several minutes. We randomly selected a train of notes emitted by a single male (128 mm snout-vent length, recorded at 20.7 °C) of average size [14]. Playback experiments were divided into three consecutive periods: 5 min silence, 5 min stimulus, 5 min silence. The stimulus consisted of nine trains of five bullfrog advertisement calls (6.6 s, 187.5 Hz dominant frequency, 234.4 Hz central frequency) separated by 30 s intervals. The stimulus was broadcast at a sound pressure level of 85 dB (C-weighting) measured at 1 m distance, which is equal to the mean amplitude we observed in the field, as calibrated using a portable sound pressure level meter (Instrutemp, ITDEC-4000).

We searched for calling male tree frogs from 21:00–0:00 h. Once a focal male was located, we placed the speaker at 1 m distance, 10 cm above the water, and directed towards the caller. We waited 3–5 min before initiating the experiment. We recorded calls of focal males (N = 10) using a digital audio recorder (Marantz PMD670) and a directional microphone (Sennheiser M66-K6P) placed 1 m from the caller. Focal males were separated by at least 10 m and were captured following each experiment.

Specimens were deposited in the Coleção de Anfíbios e Répteis, Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCT 11561–11571).

Acoustic analysis

The 15 min recordings of 10 individuals were examined using RavenPro 1.4 [15]. Spectrograms were constructed using 16 bit resolution, 22 Hz sampling rate, and 256 point fast Fourier transform. We displayed and counted the calls emitted in each of the three time periods and calculated the signal rate (calls/min). To measure call parameters, we randomly sampled 20 calls in each period (60 calls per individual) and estimated duration (s), inter-signal interval (time between sequential calls, s), dominant frequency (Hz), and centre frequency (Hz) [16].

Differences in call parameters were tested by ANOVA through randomisation tests treating individuals as blocks and the period as a fixed factor. We assumed the null hypothesis that any call or signal rate of a given individual could be emitted in any period and used 1000 permutations and pseudo-Fratio statistics as test criteria [17]. To determine which periods differed, we used pairwise contrasts calculated using only the vectors and sample units of the groups involved in the pair under test and therefore not requiring correction of P-values. We used Multiv 2.4.2 [18] for all statistical tests.

3. Results

Bullfrog calls had no effect on tree frog signal rate ($F = 0.004$, $p = 0.968$) or inter-call interval ($F = 0.004$, $p = 0.289$), but signal duration was affected by the stimulus ($F = 0.021$, $p = 0.002$; Fig. 1). Signal duration did not differ significantly between pre-stimulus and stimulus periods ($F = 0.001$, $p = 0.522$) but was significantly shorter in post-stimulus than pre-stimulus ($F = 0.018$, $p = 0.013$) and stimulus periods ($F = 0.025$, $p = 0.002$).

Bullfrog calls caused tree frogs to call at higher frequencies (Fig. 2), with a significant effect on the dominant and centre frequencies of tree frog calls ($F = 0.030$, $p = 0.001$; $F = 0.048$, $p = 0.001$, respectively). Relative to the pre-stimulus period, the dominant frequency increased during the stimulus ($F = 0.037$, $p = 0.001$) and then decreased but remained significantly higher than in the pre-stimulus period ($F = 0.021$, p

= 0.004). The difference between the dominant frequencies of the stimulus and post-stimulus periods was not significant ($F = 0.004$, $p = 0.204$). Centre frequencies differed only during the stimulus ($F = 0.048$, $p = 0.001$), being significantly different from both the pre- ($F = 0.055$, $p = 0.001$) and post-stimulus periods ($F = 0.061$, $p = 0.001$).

4. Discussion

In this study, we have demonstrated that acoustic invaders can affect native species in the acoustic niche, causing changes in the spectral properties of signals used to attract mates and repel rivals. Previous studies of responses to other kinds of environmental sounds reported significant alterations in signal rate [8–10]. In contrast, we found the signal rate of native tree frogs to be unaffected by invasive bullfrog calls. Instead, tree frogs immediately shifted calls to significantly higher frequencies. A similar spectral shift was reported for two Australian frogs in response to long-term exposure to traffic noise [19]. Such altered frequencies could be energetically suboptimal [20], which might explain the decrease in signal duration after exposure to bullfrog calls. Insofar as the advertisement call is the primary basis of mate selection [21,22], such changes could negatively impact the reproductive success of native species.

The mass of amplexant white-banded tree frogs is significantly correlated, which suggests that females choose males of proportional size [22]. This choice is probably guided by the dominant frequency of the male's advertisement call, which, as in many other anurans, is inversely proportional to body mass [11]. Given that tree frogs increase call frequencies in response to bullfrog calls, the resulting advertisement calls could affect pair formation by providing false cues of male condition.

In light of the immediate response of calling tree frogs to a simulated invasion of a single bullfrog, full-scale acoustic invasions, which often involve choruses of up to

five bullfrogs (CB, personal observation), are likely to have major impacts on the structure of acoustic communities. The effects of bullfrog vocalizations on acoustic communities are expected to be especially severe due to their broad frequency band, which masks the calls of multiple species simultaneously. Biological invasions involve diverse interactions between native and invasive species, which makes it difficult to identify the causes of alterations in native species and communities. Simulated acoustic invasions allow the effects of invasive species on native individuals and whole communities to be rigorously tested by allowing researchers to experimentally isolate and control variables in natural environments.

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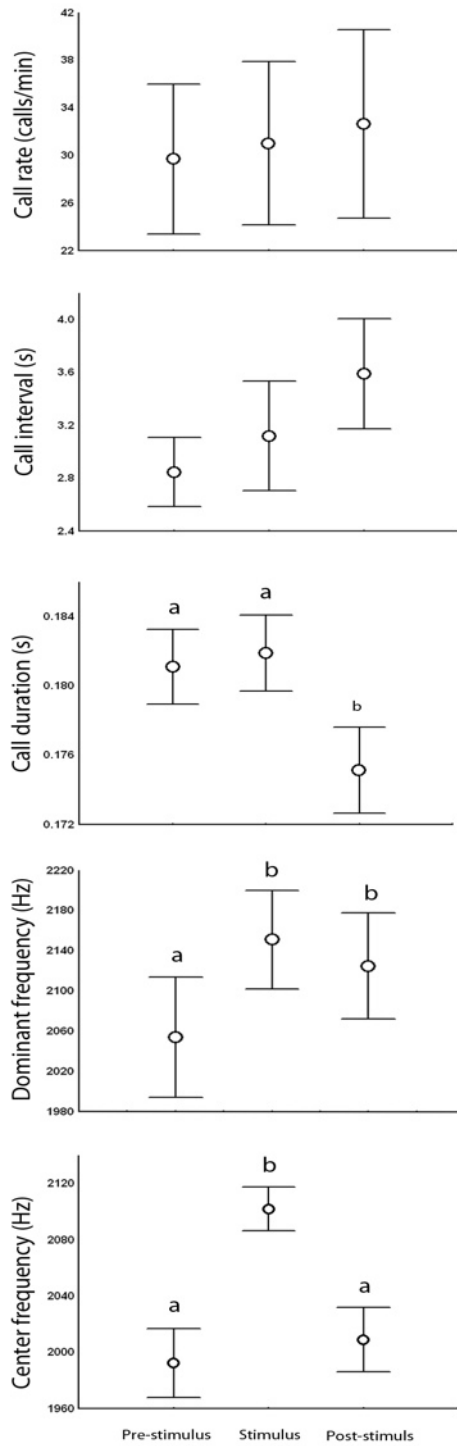


Figure 1 - Main effects (means and standard errors) of American bullfrog advertisement calls on white-banded tree frog advertisement calls. During the stimulus period, tree frogs increased the dominant and centre frequencies. During the post-stimulus period, call duration decreased while the dominant frequency remained higher than in the pre-stimulus period. Letters indicate groups that are statistically different from one another.

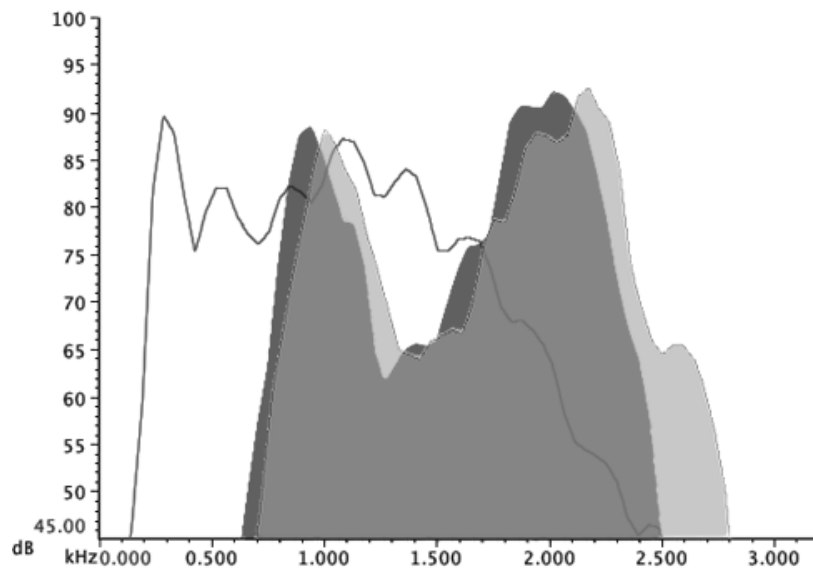


Figure 2 - Power spectra (256 FFT) of a broadcast American bullfrog advertisement call (white), and white-banded tree frog calls emitted before (dark grey) and during (pale gray) the stimulus period. Tree frog calls recorded at 22 °C; male snout-vent length 53.5 mm.

8. Conclusões gerais

8.1. Sobre as origens desta tese

Antes de resumir as conclusões apresentadas em cada um dos capítulos desta tese, eu acho relevante apresentar as suas origens. Nesta seção, eu vou narrar brevemente os acontecimentos relacionados com as minhas experiências com a rã-touro, seguindo a ordem cronológica dos acontecimentos. Se o leitor preferir, poderá iniciar a leitura diretamente na seção 8.2.

Era primavera de 2002, eu havia acabado de me tornar estagiária do laboratório de herpetologia da Universidade Federal de Santa Maria (UFSM) sob orientação da profa. Sonia Cechin. Estava ávida por começar a trabalhar, por ir para o campo, por aprender. A minha primeira expedição para campo foi para a região da Quarta Colônia no Rio Grande do Sul, especificamente a região de entorno da Usina Hidrelétrica de Dona Francisca, quando fui acompanhar as atividades de coleta de rã-touro para o mestrado de Rubens Alexandre Boelter, o Rubinho. O Rubinho tinha por objetivo analisar o conteúdo estomacal dessas rãs.

Uma vez no campo, observei os colegas passando rede de arrasto em açudes ou observando rãs — gigantes, diga-se de passagem — no banhado, e eram muitas. Nas redes, também vinham muitos girinos da espécie, em uma das poças, eu contei mais de 500 girinos. Esse número por si só não é significativo, algumas vezes já contei mais de 2000 girinos de uma única espécie em uma poça, mas é especialmente significativo para mim: eu contei os girinos, um por um, movendo-os da rede com um pinção. Se o meu leitor é um herpetólogo, a essa altura já estará rindo. Para os demais leitores, eu explico aqui que o motivo lógico para que eu movesse girino por girino com a pinça é porque eu tinha medo. Esse medo, de anfíbios em geral, fui vencendo aos poucos nos próximos

meses, porém essa história ficou (restritamente) famosa. E dessa expedição campo, o que me marcou foi o choque de ver tantas rãs EXÓTICAS, INVASORAS,, aqui!

Cinco anos depois, como aluna de mestrado de Ecologia na Universidade Federal do Rio Grande do Sul (UFRGS), eu cursava a disciplina de Ecologia Terrestre, ministrada pelo Prof. Andreas Kindel. Como o próprio Andreas vai concordar comigo, a disciplina deveria se chamar Ecologia da Conservação, pois, na verdade, discutíamos temas como fragmentação da paisagem, distúrbios e invasões. Nessa ocasião, fomos estimulados a discutir Mark Sagoff (2005): “*Do non-native species threaten the natural environment?*”. Resumindo em poucas linhas, Sagoff argumenta que o conceito de “dano” é muito questionável, e se não conseguimos precisamente definir dano ou prever o comportamento de espécies introduzidas, teríamos que barrar todas as invasões, o que seria potencialmente impossível. Segundo a leitura de Sagoff, espécies não-nativas geralmente fazem crescer a riqueza local, e poucas vezes implicam em diminuição de riqueza.

Repentinamente, eu vi os potenciais efeitos negativos de espécies invasoras sobre comunidades naturais sendo questionados, seriamente questionados. Eu, que comecei na biologia vendo tantas rãs-touro gigantes, sempre assumindo espécies invasoras como um problema, de imediato, não consegui digerir o tema da aula. Lendo Sagoff, eu tive que dar razão aos seus questionamentos, pois, tomando a invasão da rã-touro no Brasil como exemplo, eu não conseguia pensar em um estudo mostrando efeitos da espécie sobre as comunidades nativas. Pior, muito pior que isso, eu mal conseguia citar um estudo mais básico que descrevesse os padrões espaciais da invasão. A seguir, eu transcrevo o trecho final do meu trabalho na disciplina de Ecologia terrestre, no qual discuti com os artigos de Sagoff.

“...Concordo plenamente com o autor que ainda não temos conhecimento técnico e acadêmico suficiente para avaliar muitos dos casos, e estamos discutindo no nível político. Precisamos de experimentos que possibilitem melhores conclusões. Uma sugestão seria introduzir, em área controlada, duas espécies novas, sendo uma de uma localidade próxima e outra de local geograficamente distante, sendo elas filogeneticamente próximas, e acompanhar o tipo de relações que elas desenvolvem na comunidade ao longo do tempo (no mínimo 10 anos). Ainda assim, esse seria apenas mais um caso. Cada espécie, com todas as suas peculiaridades, tem uma história natural ecológica/evolutiva a ser considerada. Forçar uma evolução em curto espaço de tempo, embora possa não acarretar em grandes alterações, produz resultados imprevisíveis. Enquanto se debate o tema, parecemos verificar a globalização também da biota, mas isso pode ser bom: já vestimos roupas parecidas em grande parte do ocidente, porque não vemos as mesmas plantas e animais predominando por aí?”

Vou me abster de comentários sobre a minha ingenuidade, mas a partir desse momento, eu comecei a me interessar pelo tema, comecei a ler os trabalhos sobre a invasão de *Rhinella marina* na Austrália, e a acompanhar os trabalhos do grupo de pesquisa do prof. Richard Shine. Esta tese nasceu no momento em que percebi que algo que eu tinha como verdade — espécies invasoras são necessariamente um problema — não tinha sustentação em estudos científicos. O engraçado é que quanto mais eu fui estudando sobre invasões, mais fui entendendo a validade dos questionamentos de Mark Sagoff. O resultado disso, resumiu bem a profa. Sonia Cechin: “*tu começou a carreira contando rã-touro, e vai acabar contando rã-touro*”. Não sei se eu vou acabar contando rãs, mas vim da iniciação científica ao doutorado contando rãs.

As idéias de Sagoff receberam destaque no *Fifty Years of Invasion Ecology*, Johan Hattingh (2011) discute o “*Mark Sagoff’s challenge*”, se referindo às idéias da linha filosófica e ética que Mark Sagoff representa. Hattingh menciona inúmeros problemas

nessa abordagem, mas reconhece a importância das críticas de Sagoff para o crescimento e consolidação da biologia da invasão. Precisamos definir o que estudamos, melhorar os conceitos e sermos mais criteriosos quando falamos em invasões, respectivas implicações e, particularmente, quando direcionamos dinheiro público para prevenir ou manejar invasões.

8.2 Resultados do trabalho

Na apresentação e decorrer da tese, eu indiquei estudos-chave sobre a invasão da rã-touro no mundo, e especificamente no Brasil. Na Figura 1, eu situo alguns desses estudos e incluo os resultados dos dois primeiros capítulos desta tese na estrutura teórica para discussão de invasão proposta por Blackburn et al. (2011). Assim, é possível observar que os resultados aqui apresentados são um importante avanço na compreensão da invasão da rã-touro. Até o presente momento, estudos de história natural de populações invasoras e algumas observações já haviam nos mostrado que a espécie tinha i) sido transportada, ii) introduzida na natureza, iii) sobrevivia em populações naturais. Nesta pesquisa, nós observamos que os registros continuam aumentando e que as populações se reproduzem e/ou ocorrem em baixas abundâncias em sítios menos favoráveis, em grandes regiões da Mata Atlântica, há pelo menos mais de uma década — e provavelmente mais de duas. Esses resultados evidenciam uma invasão nos seus estágios finais, nos quais a espécie já venceu diversas barreiras ambientais. Importantes estudos já indicavam que esse cenário era provável, entretanto os trabalhos desta tese estão alertando que, ao menos na Mata Atlântica sul do Brasil, ele já é uma realidade.

Uma vez que nós apresentamos uma invasão de grandes proporções — e foi tão fácil selecionar 90 corpos d'água para o estudo, e a rã-touro foi observada em quase 70 deles — é muito difícil pensar em erradicação, como proposto por alguns colegas. No

capítulo II, nós tangenciamos o tema de manejo de rã-touro e fomos cautelosos em afirmar que ainda que não temos evidência de impacto, devemos ser precavidos e focar atenção para áreas prioritárias.

A minha opinião pessoal é a de que a questão do manejo merece uma nova tese de doutorado, testando métodos apropriados, e os estudos testando potenciais efeitos devem continuar. Nos capítulos III e IV, nós testamos potenciais impactos da invasão da rã-touro. No capítulo III, o objetivo principal era testar os potenciais efeitos negativos da invasão da rã-touro sobre a riqueza de anfíbios nativa, considerando potenciais descritores espaciais e ambientais que podem estruturar essa relação. Os resultados não suportaram nenhum efeito negativo da abundância das populações da rã-touro sobre a riqueza de anfíbios nativos. Ao contrário, o modelo linear simples revelou uma relação fraca e positiva, que o modelo causal revelou ser uma relação indireta. A rã-touro foi mais abundante em algumas composições de comunidades e variou de forma similar a espécies comuns, como *Dendropsophus minutus* e *Physalaemus cuvieri*, e essas comunidades podem ser ricas em espécies. Entretanto, foi salientado no artigo e eu saliento aqui novamente que, em menores abundâncias, a espécie está co-ocorrendo com diversas espécies, e nós estamos longe de poder dizer que populações invasoras da rã-touro não causam impacto nenhum sobre as comunidades nativas, ou não causarão no futuro.

A continuidade dos trabalhos, enfocando potenciais efeitos da rã-touro sobre as comunidades exclusivamente aquáticas, como anfíbios na fase larval, peixes e invertebrados, pode revelar padrões diferentes dos apresentados nesta tese. Efeitos de predação e competição podem ser maiores sobre organismos que estão espacialmente restritos a um dado habitat, como uma poça.

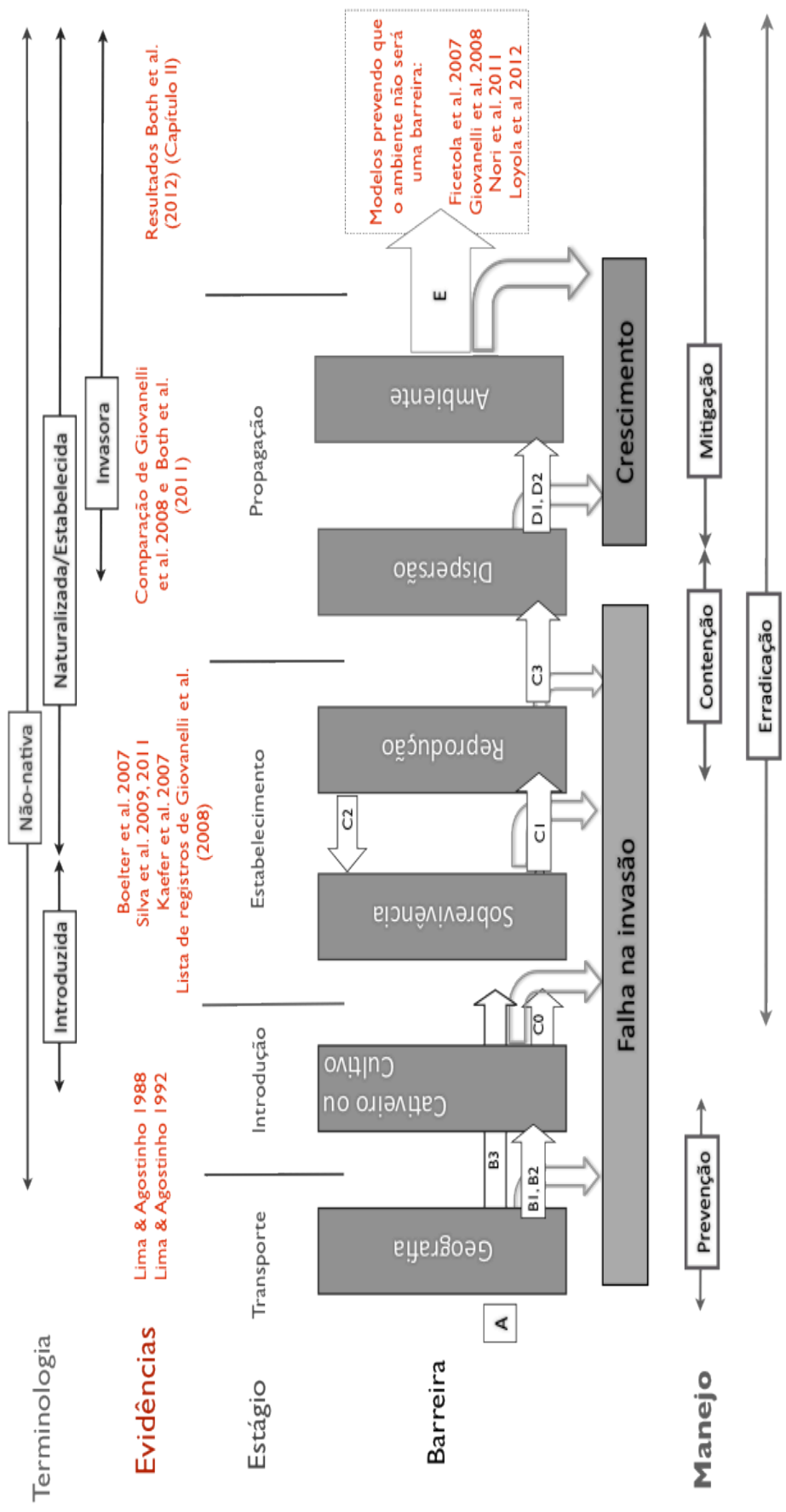


Figura 1 - Evidências fornecidas por estudos realizados com a biologia da rã-touro no país para a classificação do estágio da invasão. Os estudos de modelagem de nicho para a espécie foram diferenciados dos demais uma vez que seus resultados reportam previsões. * Acompanham evidências fornecidas por trabalhos dessa tese.

Nós testamos um novo mecanismo pelo qual a invasão pode afetar espécies nativas da comunidade no capítulo IV. Nesse capítulo, o objetivo era testar se a rã-touro promove competição no nicho acústico com espécies nativas, avaliando a influência do canto de anúncio da rã-touro sobre uma espécie nativa. Testamos os efeitos do canto da rã-touro sobre *Hypsiboas albomarginatus* em um experimento de curta duração, que foi suficiente para causar mudanças no canto da espécie.

Com certeza teremos que testar, no futuro, se essas mudanças se refletem em redução de *fitness*. Pode ser que, a longo prazo, as espécies se adaptem e a mudança não afete as populações. Entretanto, é possível que coros de quatro, cinco, seis, ou até nove machos (como observou Camila Medeiros descrevendo a fenologia reprodutiva da espécie no sul do Brasil) tenham um efeito importante sobre as comunidades acústicas.

A origem desse capítulo é diferente da dos demais; ele não estava no meu projeto original. A idéia surgiu pela primeira vez enquanto eu estava na minha mesa pensando em como introduzir girinos de rã-touro em ambiente natural, de forma segura e controlada. Essa proposta tinha a finalidade de observar a competição e a influência da estrutura trófica sobre a potencial competição em um ambiente real não-invadido, pois condições experimentais de sistemas simples, como aquários e poças artificiais, poderiam apresentar relações mais intensas do que aquelas que ocorrem na natureza. Uma gaiola também poderia forçar essas relações, mas alguma heterogeneidade ambiental estaria incorporada. Contudo, eu teria ainda que selecionar girinos e testar se não estavam infectados com *Batrachochytrium dendrobatidis*, e praticamente “acreditar” que não estariam carregando outros microorganismos indesejáveis. Também teria que assegurar 100% de eficiência das gaiolas em não permitir nenhum escape. Ou seja: era quase uma “missão”.

Então eu pensei em modelinhos de madeira, para ver o deslocamento espacial que um anuro tão grande poderia causar em machos cantores de outras espécies, simplesmente por estar ali. Considerei a ideia simples demais e de repente pensei: o que aconteceria se eu introduzisse o som da rã-touro, apenas o som? Seguiu-se uma olhada rápida no Google. Logo, fui conversar com o meu orientador, prof. Taran Grant. Quando eu comentei a ideia, ele não deu muita atenção, mas acho que bastaram algumas horas dentro da mesma tarde para ele achar isso “o máximo”. Nos próximos dias, nós fomos dando corpo a esse experimento.

Eu considero só o começo, temos muito para saber sobre a invasão acústica e os seus efeitos. Como eu citei na apresentação da tese, o *Eleutherodactylus coqui*, por exemplo, causa impacto no mínimo no mercado imobiliário do Hawaii (Beard et al. 2009). Podemos pensar sobre a invasão do nicho acústico para diversas espécies, diversos grupos, e também podemos pensar que sons invasores não necessariamente requerem espécies invasoras. As pesquisas sobre os efeitos do barulho antropogênico sobre comunidades são pesquisas sobre a invasão do nicho acústico. As ondas sonoras produzidas por equipamentos que utilizamos podem ser vistas como “entidades acústicas” que introduzimos na beira das matas e no fundo dos mares. Tomando com exemplo as nossas residências, podemos suportar certos graus de ruídos, em certas frequências. Todavia, os efeitos de alguns sons e frequências podem ser adversos.

8.3. *Últimas considerações*

Nós apresentamos só o começo dos nossos estudos sobre esse tema, mas eu saliento que ainda temos muito por conhecer em invasão, ou, como disse Richardson (2011), caminhamos uma longa estrada, mas há muito por caminhar. Temos mais por revelar, mesmo para uma espécie tão estudada no mundo todo como a rã-touro. Nós

temos mais análises por fazer e muito por conhecer. Elton, em 1958, já nos alertava que as invasões cresceriam junto com os distúrbios e com a crescente mobilidade humana. Simberloff & Von Holle, em 1999, no primeiro volume da recém-nascida *Biological Invasions*, já previam que invasões facilitariam novas invasões. E nós vivemos num mundo que produz alimento, vestuário e renda cultivando espécies exóticas, que talvez serão ainda mais beneficiadas pela degradação e mudanças climáticas futuras. Logo: temos que nos tornar hábeis em lidar com essa realidade! Estamos muitos anos aquém nas pesquisas se considerarmos os patamares das nossas necessidades.

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8. Normas para publicação

9.1 South American Journal of Herpetology

INSTRUCTIONS TO AUTHORS

(April 2008)

General Information

The South American Journal of Herpetology (SAJH) is an international journal, published by the Brazilian Society of Herpetology, that aims to provide an effective medium of communication for the international herpetological community. SAJH publishes original contributions on the Biology of Amphibians and Reptiles, covering primarily the fields of systematics, paleontology, evolutionary biology, ecology, natural history, taxonomy, behavior, anatomy, functional morphology, comparative morphology and physiology, ecophysiology, genetics, molecular biology, ontogeny, biogeography, and conservation biology. SAJH also encourages submission of eminently theoretical studies that explore principles and methods on these fields.

All contributions must follow the International Code of Zoological Nomenclature and relevant specimens should be properly curated and deposited in a recognized public or private, non-profit institution. Authors should observe appropriate ethical and legal guidelines and regulations for any study that involve live organisms. Therefore authors are encouraged to indicate in the acknowledgment section proper collecting permits and protocols approved for field and laboratory researches. Tissue samples should be referred to their voucher specimens and all nucleotide sequence data (aligned as well as unaligned) should be submitted to Genbank (<http://www.ncbi.nlm.nih.gov/genbank/>) or EMBL (<http://www.ebi.ac.uk/>) shortly after publication.

Submission of Manuscripts

All the submission and review process is done electronically. Thus manuscripts should be sent electronically only to the Editor (Hussam Zaher, editor@sbherpetologia.org.br).

Manuscripts are considered on the understanding that they have not been published and/or will not appear elsewhere in substantially the same or abbreviated form. The criteria for acceptance of articles are quality and relevance of research, clarity of text, and compliance with the guidelines for manuscript preparation.

All manuscripts should be written in English, using U.S. spelling and grammar conventions, and should include an abstract in English and its translation either in Portuguese or Spanish. Text should be submitted in MS Word (*.doc), preferably for PC, and must include line numbers (**see below for specific procedure**). Artwork and photographs should be submitted as electronic files, in *.jpg format only. Manuscripts will be assigned to Associate Editors, who in turn will seek two or three reviews of each submission. Once a manuscript is accepted providing changes suggested by the referees, the author is requested to return a revised electronic version and a detailed letter addressing how reviewers' suggestions were incorporated within two weeks upon

receiving the communication by the Associate Editor. Communications regarding acceptance or rejection of manuscripts are made through correspondence with the first or corresponding author only. When the manuscript is accepted for publication, authors will be asked to provide updated electronic files of the text in ***.doc**, and relevant graphic files produced in high quality ***.jpg** file (**at least 8 cm wide with 350 dpi**). Submission of hard copies by mail will not be accepted.

No figures should be embedded in text files.

Proofs

Page-proofs with the revised version will be sent electronically to the first or corresponding author. **Page-proofs must be returned to the editor within 48 hours.** Failure to return the proof promptly may be interpreted as approval with no changes and/or may delay publication. Only necessary corrections in proof will be permitted. Once page proof is sent to the author, further alterations and/or significant additions of text are permitted only at the author's expense or in the form of a brief appendix ("note added in proof").

Manuscript Form

Authors are requested to pay close attention to the instructions concerning the preparation

of manuscripts. Close adherence to the guidelines will expedite processing of the manuscript, whereas manuscripts deviating from the required form **will be returned for revision prior to review.**

Manuscripts should not exceed 30 pages of double-spaced typescript on 21 x 29.7 cm (A4 format) paper (Letter format will not be accepted), with wide margins (2.5 cm). The pages of the manuscript should be numbered consecutively. The text must include **line numbers, which are activated through the "Page Setup" command of the "File" menu in a Word document (go to "Layout" and activate "Line numbers")**. Scientific names should appear in italics. The article should be arranged in the following order: title page, abstracts, body of text, literature cited, appendices, tables and figure captions, tables, and figures. Each of these sections should begin on a new page. All typescript pages must be double-spaced.

Title page. This should include the title with all letters capitalized, author(s) name(s) in small caps, their address(es) (including electronic address), and between 3-8 Key words in English. Multiauthors manuscripts must identify a corresponding author and address. The title should be concise and, where appropriate, should include mention of families and/or higher taxa. Names of new taxa should not be included in titles.

Example:

How to be arboreal and diurnal and still stay alive: microhabitat use, time of activity, and defense in Neotropical forest snakes

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4 Corresponding author.

Key words: Defense; Habitat; Amazonia; Atlantic Forest; Brazil.

Abstract page. All papers should have an abstract in English followed by its translation in Portuguese or Spanish. The abstract is of great importance as it may be reproduced elsewhere. It should be in a form intelligible if published alone and should summarize the main facts, ideas, and conclusions of the article. Indicative abstracts are strongly discouraged. Include all new taxonomic names for referencing purposes. Abbreviations should be avoided. It should not include references.

Body of text. The main body of the text should include the following sections: Introduction, Material and Methods, Results, Discussion, and Acknowledgments. Primary headings in the text should be in capital letters and centered; the following text should begin on the next line, indented. Secondary headings should be in capital and lowercase letters and flush left; the following text should begin on the next line, indented. Tertiary headings should be in capital and lower case letters, in italics and indented; the following text should be on the same line and separated from the heading by a hyphen.

Literature cited. Citations in the text should be arranged in chronological order first and alphabetical order second. Citation in text should be given as: Silva (1998)..., Silva (1998: 14-20)..., Silva (1998: figs. 1, 2)..., Silva (1998a, b)..., Silva and Oliveira (1998) ..., (Silva, 1998)..., (Rangel, 1890; Silva and Oliveira, 1998a, b; Adams, 2000)..., (H. R. Silva, pers. comm.)..., (Silva et al., 1998), the latter when the paper has three or more authors. The reference need not be cited when author and date are given only as authority for a taxonomic name. The literature section should be arranged strictly alphabetically and given in the following format:

Journal Article – Silva, H. R., H. Oliveira, and S. Rangel. Year. Article title. Journal name, 00: 000-000. Journal names must be fully spelled out.

Books – Silva, H. R. Year. Book title. Publisher, Place, 000 pp.

Articles in Books – Silva, H. R. Year. Article title; pp. 000-000. In: H. Oliveira and S. Rangel (Eds.), Book Title. Publisher, Place.

Articles in Larger Works – Silva, H. R. Year. Article title; pp. 000-000. In: H. Oliveira and S. Rangel (Eds.), Title of Larger Work. Serial Publication 00. Publisher, Place.

Dissertations and Theses – Silva, H. R. Year. Dissertation title. Ph.D. dissertation, University, Place, 000 pp.

Appendices, Tables, Figures, Tables and Figure captions. These should be arranged sequentially after the literature section of the manuscript, in the following order: Appendices, table and figure captions (together in the same section), tables, and figures. Each of these sections should begin in a new page.

Appendices. Appendices should be numbered using Roman numerals and must be numbered in the same sequence in which they appear in the text where they are referred as Appendix I, Appendix I-IV, etc. Use “APPENDIX” in the appendix heading.

Tables. Tables should be on separate pages and they must be numbered in the same sequence in which they appear in the text. Authors are encouraged to indicate where the tables should be placed in the text. They should be comprehensible without reference to the text. Tables should be formatted with horizontal, not vertical, rules. In the text, tables should be referred as Table 1, Tables 2 and 3, Tables 2-6. Use “TABLE” in the table heading.

Illustrations/Photographs. The number and size of the illustrations must be kept to the minimum required for clarification of the text. Previously published figures cannot be accepted. Figures should be on separate pages and numbered consecutively in the same sequence they appear in the text. Figure captions should be on a separate page preceding the figures. Separate illustrations of a composite figure should be identified by capital letters and referred in the text as so (Fig. 1A). Where possible, letters should be placed in the lower right corner of each illustration of a composite

figure. Hand-written lettering on illustrations are unacceptable. A scale bar should be marked on each figure so that absolute sizes are clearly apparent and any reduction is automatically compensated for. The length of the scale bar should be stated in the caption. On no account should magnification factors (e.g. x7000; x80000) be expressed in the captions. SAJH accepts only figures in electronic version. Hard copies of figures sent by mail will not be accepted. Figures should be submitted in easy to load files in *.pdf, *.ppt or *.jpg format. Final figures should be sent to the

Editor in high quality electronic formats only (*.jpg or *.tif) with at least 8 cm wide and 350 dpi. Figures should be mounted in order to minimize blank areas between separate illustrations. Costs for printing high quality color figures will be in charge of the author (s). For referring to figures in the text, authors should use “(Fig(s).)” and “Figure(s)”, but “FIGURE(S)” in the figure captions and “(fig(s).)” when referring to figures in another paper. Authors are encouraged to indicate where the figures should be placed in the text.

9.2 Journal of Applied Ecology

Manuscript Structure

STANDARD PAPERS. Original articles should not exceed 7000 words inclusive of all parts of the paper apart from online Supporting Information. Typescripts should be arranged as follows, with each section starting on a separate page.

Title page. This should contain:

- A concise and informative title.
- A list of author names, affiliation(s), and e-mail addresses.
- The name, complete mailing address (including e-mail address, telephone and fax numbers) of the corresponding author.
- A running title not exceeding 45 characters.
- A word count of the entire paper broken down into summary, main text, acknowledgements, references, tables and figure legends.
- The number of tables and figures.
- The number of references.

Summary. This is called the Abstract on the web submission site. The Summary should outline the purpose of the paper and the main results, conclusions and recommendations, using clear, factual, numbered statements. Authors should follow a formula in which point 1 sets the context and need for the work; point 2 indicates the approach and methods used; the next 2-3 points outline the main results; and the last point identifies the wider implications and relevance to management or policy. The final summary point must carry the subheading '***Synthesis and applications***' and is the most important of all in maximising the impact of the paper. It should synthesise the paper's key messages and should be generic, seminal and accessible to non-specialists. The whole Summary should be readily understandable to all the Journal's readers and must not exceed 350 words.

Keywords. A list in alphabetical order not exceeding ten words or short phrases, excluding words used in the title and chosen carefully to reflect the precise content of the paper.

Introduction. State the reason for the work, the context, background, aims and the hypotheses being tested. End the Introduction with a brief statement of what has been achieved.

Materials and methods. Include sufficient details for the work to be repeated. Where specific equipment and materials are named, the manufacturer's details (name, city and country) should be given so that readers can trace specifications by contacting the manufacturer. Where commercially available software has been used, details of the supplier should be given in brackets or the reference given in full in the reference list.

Results. State the results of experimental or modelling work, drawing attention to important details in tables and figures. The Results section should conform to the highest standards of rigour.

Discussion. Point out the importance of the results and place them in the context of previous studies and in relation to the application of the work (expanding on the Synthesis and applications section of the Summary). Include clear recommendations for management or policy.

Acknowledgements. Be brief. If authors refer to themselves as recipients of assistance or funding, they should do so by their initials separated by points (e.g. J.B.T.). Do not acknowledge Editors by name.

References (see Manuscript Specifications below).

Tables (see Specifications). Each table should be on a separate page, numbered and accompanied by a legend at the top. These should be referred to in the text as Table 1, etc. Avoid duplication between figures and tables.

Figures (see Specifications). Figures and their legends should be grouped together at the end of the paper before Supporting Information (if present). If figures have been supplied as a list at the end of the text file (as recommended), they should appear above their respective legend. Figures should be referred to in the text as Fig. 1, Figs 1 & 2, etc. Photographic material should also be referred to as Figures. Do not include high-resolution versions of figures at submission; reduce the size and resolution of graphics to a file size of less than 1 MB. If a manuscript is accepted, higher quality versions of figures can be submitted at a later stage.

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In order to promote the advancement of science through the process of documenting and making available the research information and supporting data behind published studies, the editors of this journal strongly encourage authors to make arrangements for archiving their underlying data.

REVIEWS. Reviews should not exceed 8000 words inclusive of all parts of the paper. The layout should follow the same format and specifications as for Standard Papers except that the organisation of the main text need not follow the division into Introduction, Materials and methods, Results and Discussion.

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LANGUAGE. Manuscripts must be written in English. They should be clear, concise and grammatically correct. Spelling should conform to the *Concise Dictionary of Current English*. Journal style is not to use the serial comma (also known as the Oxford or Harvard comma) before and/or/nor unless meaning would otherwise be obscured. Editors reserve the right to modify accepted manuscripts that do not conform to scientific, technical, stylistic or grammatical standards, and minor alterations of this nature may not be seen by authors until the proof stage.

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SCIENTIFIC NAMES. Give Latin names in full, together with the naming authority, at first mention in the main text. Subsequently, the genus name may be abbreviated, except

at the beginning of a sentence. If there are many species, cite a Flora or check-list which may be consulted for authorities instead of listing them, in the text. Do not give authorities for species cited from published references. Give priority to scientific names in the text (with colloquial names in parentheses if desired). Latin names following common names should not be separated by a comma or brackets.

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UNITS, SYMBOLS AND ABBREVIATIONS. Authors should use the International System of Units (S.I., Systeme International d'Unités; see *Quantities, Units and Symbols*, 2nd edn (1975) The Royal Society, London). Mathematical expressions should contain symbols not abbreviations. If the paper contains many symbols, they should be defined as early in the text as possible, or within the Materials and methods section. Journal style for time units are: s, min, h, days, weeks, months, years. Use 'L' for litre not 'l' to avoid confusion with 'one'. Use the negative index for units, e.g. number of insects g⁻¹ dry wt (also note there is no period for wt). Probability values should be denoted as *P*.

MATHEMATICAL MATERIAL. Mathematical expressions should be carefully represented. Wherever possible, mathematical equations and symbols should be typed in-line by keyboard entry (using Symbol font for Greek characters, and superscript options where applicable). Do not embed equations or symbols using Equation Editor or Math Type, or equivalents, when simple in-line, keyboard entry is possible. Equation software should be used only for displayed multi-line equations, and equations and symbols that cannot be typed. Suffixes and operators such as d, log, ln and exp will be set in Roman type: matrices and vectors will be set in italic. Make sure that there is no confusion between similar characters like l ('ell') and 1 ('one'). Ensure that expressions are spaced as they should appear. If there are several equations they should be identified by an equation number (i.e. 'eqn 1' after the equation, and cited in the text as 'equation 1').

NUMBER CONVENTIONS. *Text:* Numbers from one to nine should be spelled out except when used with units, e.g. two eyes but 10 stomata; 5 °C, 3 years and 5 kg. *Tables:* Do not use excessive numbers of digits when writing a decimal number to represent the mean of a set of measurements. The level of significance implied by numbers based on experimental measurements should reflect, and not exceed, their precision; only rarely can more than 3 figures be justified. Be consistent within tables.

FIGURES (INCLUDING PHOTOGRAPHS). Please follow the instructions on figure format and content carefully to avoid delays in manuscript processing. All illustrations are classified as figures.

Figures should be placed at the end of the document and each must have a legend, presented separately from the figure. The legend should provide enough detail for the figure to be understood without reference to the text. Information (e.g. keys) that appear on the figure itself should not be duplicated in the legend. In the full-text online edition of the Journal, figure legends may be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should inform the reader of key aspects of the figure.

Figures should be drawn to publication quality and to fit into a single column width (71 mm) wherever possible. To make best use of space, you may need to rearrange parts of figures. If figures are prepared that will require reduction, please ensure that axes, tick marks, symbols and labels are large enough to allow reduction to a final size of about 8 point, i.e. capital letters will be about 2mm tall. Figures should not be boxed and tick marks should be on the inside of the axes. Lettering should use a sans serif font (e.g. Helvetica, Arial) with capitals used for the initial letter of the first word only. Bold lettering should not be used. Units of axes should appear in parentheses after the axis name. All lettering and symbols must be proportioned, clear and easy to read, i.e. no labels should be too large or too small. Label multi-panel figures (a), (b), (c), etc., preferably in the upper left corner. Use greyscales (e.g. 0, 20, 40, 60, 80, 100%) in preference to pattern fills where possible. If colour figures are submitted for colour online publication only, ensure that after conversion to greyscale they remain entirely intelligible for the black-and-white print publication of your paper. Full instructions on preparing your figures are available [here](#).

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File formats. At the time of submission, or after acceptance of the manuscript for publication, figure files should be supplied as follows. Photographic figures should be saved in tif format at 300 d.p.i. (or failing that in jpg format with low compression) and should have good contrast. Line figures should be saved as vector graphics (i.e. composed of lines, curves, points and fonts; not pixels) in pdf, eps, ai, svg or wmf format, or embedded as such in Word, as this enhances their display when published online. Combination figures (those composed of vector and pixel/raster elements) should also be saved in pdf, eps, ai, svg or wmf format where possible (or embedded as such in Word). If line figures and combination figures cannot be saved in vector graphics format, they should be saved in tif format at high resolution (i.e. 600 d.p.i.) (do not save them in

jpg format as this will cause blurring). If you are unsure about the quality of your figures, please inspect a small portion by zooming in to check that fonts, curves and diagonal lines are smooth-edged and do not appear unduly blocky or burred when viewed at high magnification. Note that line and combination figures supplied in tif format are downsampled for online publication, authors should therefore preferentially opt for vector graphic formats for these figure types (note, however, that for print publication full resolution files will be used). For full instructions on preparing your figures please refer to our **Electronic Artwork Information for Authors** page.

TABLES. Tables should be constructed using 'Tabs' rather than spaces or software options. Units should appear in parentheses after the column or row title, e.g. Time (days). Each table should be on a separate page, numbered and titled, and included at the end of the paper before the figures. The table caption must appear above the table and must NOT end in a full stop. Table footnotes should be indicated using symbols *, †, ‡, ¶, § (not superscripted); these should be doubled-up if more than 5 are needed (**, ††, ‡‡, ¶¶, §§), or if more than 10 are needed use superscript letters a, b, c, etc., throughout. References to tables in the text should not be abbreviated, e.g. Table 1.

CITATIONS AND REFERENCES. Citation to work by four or more authors should be abbreviated with the use of *et al.* (e.g. Manel *et al.* 1999). Citation to work by one, two or three authors should always give the author names in full. Work with the same first author and date should be coded by letters, e.g. Thompson *et al.* 1991a,b. Citations should be listed in chronological order in the text and be separated by a semi-colon, e.g. Balmford & Gaston 1999; Royle *et al.* 2007. The references in the Reference list should be in alphabetical order with the journal name unabbreviated. The format for papers, theses, entire books and chapters in books is as follows:

Begon, M., Harper, J.L. & Townsend, C.R. (1996) *Ecology: Individuals, Populations and Communities*, 3rd edn. Blackwell Science, Oxford.

Tuytens, F.A.M. (1999) *The consequences of social perturbation caused by badger removal for the control of bovine tuberculosis in cattle: a study of behaviour, population dynamics and epidemiology*. PhD thesis, University of Oxford.

McArthur, W.M. (1993) History of landscape development. *Reintegrating Fragmented Landscapes* (eds R.J. Hobbs & D.A.Saunders), pp. 10-22. Springer Verlag, Berlin.

Hill, M.O., Roy, D.B., Mountford, J.O. & Bunce, R.G.H. (2000) Extending Ellenberg's indicator values to a new area: an algorithmic approach. *Journal of Applied Ecology*, **37**, 3-15.

References should be cited as 'in press' only if the paper has been accepted for publication. Work not yet submitted for publication or under review should be cited as 'unpublished data', with the author's initials and surname given; such work should not be included in the Reference section. Any paper cited as 'in press' or under review

elsewhere must be uploaded as part of the manuscript submission as a file 'not for review' so that it can be seen by the editors and, if necessary, made available to the referees.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting.

EndNote reference styles can be searched for here:

<http://www.endnote.com/support/enstyles.asp>

Reference Manager reference styles can be searched for here:

<http://www.refman.com/support/rmstyles.asp>

Citations from the world wide web: Authors may sometimes wish to cite information available from the world wide web in similar ways to the citation of published literature. In using this option, authors are asked to ensure that:

- (i) fully authenticated addresses are included in the reference list, along with titles, years and authors of the sources being cited, and the most recent date the site was accessed;
- (ii) the sites or information sources have sufficient longevity and ease of access for others to follow up the citation;
- (iii) the information is of a scientific quality at least equal to that of peer-reviewed information available in learned scientific journals;
- (iv) hard literature sources are used in preference where they are available.

It is likely that official web sites from organisations such as learned societies, government bodies or reputable NGOs will most often satisfy quality criteria.

9.3 Biological Invasions

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.

Title Page

The title page should include:

- 1 The name(s) of the author(s)
- 2 A concise and informative title
- 3 The affiliation(s) and address(es) of the author(s)
- 4 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.

Citation

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

- 1 Negotiation research spans many disciplines (Thompson 1990).
- 2 This result was later contradicted by Becker and Seligman (1996).
- 3 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.

- 1 Journal article
- 2 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
- 3 Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:
- 4 Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. *N Engl J Med* 341:325–329
- 5 Article by DOI
- 6 Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. *J Mol Med*. doi:10.1007/s001090000086
- 7 Book
- 8 South J, Blass B (2001) *The future of modern genomics*. Blackwell, London
- 9 Book chapter
- 10 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
- 11 Online document
- 12 Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. <http://physicsweb.org/articles/news/11/6/16/1>. Accessed 26 June 2007
- 13 Dissertation
- 14 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

- 1 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.

- 2 All tables are to be numbered using Arabic numerals.
- 3 Tables should always be cited in text in consecutive numerical order.

- 4 For each table, please supply a table caption (title) explaining the components of the table.
- 5 Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- 6 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

- 1 Supply all figures electronically.
 - 2 Indicate what graphics program was used to create the artwork.
 - 3 For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MS Office files are also acceptable.
 - 4 Vector graphics containing fonts must have the fonts embedded in the files.
 - 5 Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.
-
- 1 Definition: Black and white graphic with no shading.
 - 2 Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
 - 3 All lines should be at least 0.1 mm (0.3 pt) wide.
 - 4 Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
 - 5 Vector graphics containing fonts must have the fonts embedded in the files.

9.4 Biology Letters

Format requirements

Research Articles and Opinion Pieces

Research articles and opinion pieces submitted to *Biology Letters* should be no longer than 2500 words. This includes cover page, abstract, references, acknowledgements and figure/table legends. **The word count is strictly enforced.**

We encourage submission of your paper as a text file (eg .doc). Each figure should be uploaded **separately** with legends included in the main text file.

We allow a maximum of 4 displays, only 2 of which can be figures.

Biology Letters welcomes opinion pieces. If you are interested in writing an opinion piece please contact the [editorial office](#) with your proposal. An opinion piece should be written as a perspective, not a formal review. It should be of interest to a broad readership and at the cutting-edge of science. A brief review and critique of past and current work should be given, but the author's own outlook on the subject should be included, in addition to their view on new directions in the field and how it should progress. The author does not have to agree with conventional thought, but should present both sides of any debate.

A research article is the presentation of the authors own most recent work, including methods and results.

While we do not publish formal reviews, we welcome submissions of formal comparative analyses and formal meta-analyses of topics within the scope of *Biology Letters*. Both types of syntheses can often be presented succinctly without lessening their impact. Raw data for these formal analyses along with detailed analytical methods should be placed in Electronic Supplementary Material (ESM).

Comments and Invited Replies

The editor of *Biology Letters* welcomes the submission of short comments on articles published within *Biology Letters*. Once submitted, all comments will be peer-reviewed. If accepted, authors of the original article will be sent the comment and invited to submit a short reply. Comments may take longer to be published due to the subsequent request for a reply.

Comments and replies in *Biology Letters* should not exceed 1000 words and should contain no more than one display. They should be brief and comment succinctly on the earlier published work. Abstracts and keywords are not used. Otherwise, formatting conforms to standard *Biology Letters* research article format. Due to the word count, inclusion of extensive references should be limited.

Please contact the [editorial office](#) prior to submission with any queries.

Meeting Reports

Biology Letters publishes brief reports on scientific meetings. If you are interested in writing a meeting report please contact the editorial office with your proposal.

A meeting report should begin with an introduction to the field, outline the highlights of the meeting, cover major advances and give the author's perspective on emerging trends and future directions the discipline may follow. It must not be a blow by blow account of every talk and every poster at the meeting; however, it should strive to cover the whole meeting, not just focus on a single session within a meeting. The report should be of interest to scientists from across the biological sciences and not limited to those who work within the same field. In order for the report to remain topical, please submit it within 4 weeks of the meeting's conclusion.

As with research articles, the report ought not to exceed 2500 words. The report will be reviewed by the Editor-in-Chief of the journal.

Article structure

Please use **double line-spacing** as this is the preferred format of our referees.

An article typically consists of the following, with numbered main sections:

- Title
- - Authors' names and full addresses where the work was carried out, plus email address of corresponding author. (In addition to providing the addresses where the work was carried out, the current addresses, where different, should be given.)
- Summary* (maximum 200 words)
- Keywords (3-6)
- Introduction
- Material and Methods section (in small type)
- Results section
- Discussion section
- Acknowledgements
- References
- Place any figure captions or tables at the end of the typescript
- Short title (for page headings)

Please note that footnotes are not used.

***Please ensure that your summary contains all likely search terms to assist indexers (e.g. PubMed) which scan only the title and abstract of articles. If possible, it is beneficial to have all your keywords written into the abstract.**

When uploading your article, we will ask for suggested referees and your preferred board member.

References

Please note, we have recently changed the style of references we use: all articles being published online now, and in print issues from 2011 onwards, use the Vancouver referencing style. Examples of articles using this style may be found on *FirstCite*.

All references to the literature cited should be given in the order of their appearance in the text in a consecutively numbered list at the end of the article. Each reference should contain as many of the following elements as possible:

- Author surnames with initials (up to 10 before et al is used)
- Year of publication
- Title of paper (roman) or book (italic)
- Journal name (italic), using standard abbreviation
- Volume number (bold)
- First and last page numbers
- DOI

Note that for a book, the edition, the chapter(s) and its/ their page range(s), the editor(s), the place of publication (if it is not obvious) and the name of the publisher should be given, for instance:

- Falconer, D. S. 1981 *Introduction to quantitative genetics*, 2nd edn. London: Longman.
-
- Falkenmark, M. 1993 *Landscape as life support provider: water-related limitations*. In *Population-the complex reality* (ed. F. Graham-Smith), pp. 103-116. London: The Royal Society.
- Nilsson, L. A. 1988 The evolution of flowers with deep corolla tubes. *Nature***334**, 147-149.
- References in the text are listed according to the Vancouver style (not by name and date), i.e. numbered consecutively in the order they appear in the text. Text citations are Arabic numerals in square brackets, for instance:
- This action has been described frequently elsewhere [1-3, 6].
- Authors are encouraged to quote digital object identifiers (DOIs) - standardized article reference codes - where known, in addition to providing full citations, for instance:
-
- Worden, B. D. & Papaj, D. R. 2005 Flower choice copying in bumblebees. *Biol. Lett.***1**, 504-507. (DOI 10.1098/rsbl.2005.0368)

The DOI is a unique electronic tag applied by certain publishers (and online databases, such as CrossRef) to their published papers. DOI hotlinks take a reader directly from the paper they are reading to the abstract of the paper they have selected. For more information, please see the [DOI homepage](#).

An EndNote style file is available to download.

Tables

Tables, however small, should be numbered and referred to in the text by their numbers.

Table captions should be brief, with descriptions of experimental detail given directly beneath, in parentheses.

Column headings should, wherever possible, be in lower-case type, and the units of measurement and any numerical factors should be placed at the head of each column.

Units should be contained within parentheses, eg distance (cm).

Figure requirements

All Royal Society journals require figures in electronic format. To ensure high-quality reproduction, and to prevent delays in publication, it is essential that figures be supplied in the correct format. Hand-drawn illustrations are not acceptable.

Multipart figures

Multipart figures should not be supplied as separate files, but should be laid out in one file by the author.

Dimensions

Artwork should be supplied at the size the author intends it to be reproduced.

Figures should be sized as follows:

Maximum width within single column, 84 mm.

Maximum width within double column, 175 mm.

Maximum height for both, 250 mm.

Text: Times, upright, 9pt, 11pt leading

Figure labels

Labels should be added to the original drawings before submission using lower-case lettering (Times Roman Font) wherever possible. Labels should be brief, e.g. (a), (b), and explained in the legend. Labels should be consistent, and close to the 9 point at final size. Mathematical symbols must follow the style of the text - variables should be distinguished from labels through italicization. Descriptions should be placed whenever possible in the captions and not on the figures themselves, although a key to symbols is often better placed within the body of a figure.

Figure format

The type of image(s) a figure contains determines which format it is best supplied in. Images fall into one of two categories:

- Bitmap (rasterized) images: Composed of pixels (e.g. photographs, scans)
- Line (vector) art: Composed of lines, fills and text (e.g. histograms, line drawings)

Photographs and scans should be supplied as high-resolution (300-600ppi/120-240ppc) bitmaps in one of the following formats (please note these formats are not compatible with LaTeX submissions):

- TIFF

- Adobe Photoshop

(jpeg images are only acceptable if taken with a digital camera) Also "unsampling" of images is not acceptable, i.e. once an image has been saved down to 72ppi the quality will remain poor even if resaved at 300ppi.

Line art (or images containing both line art and bitmap images) should be supplied in one of the following formats:

- Postscript (PS, EPS or PDF)
- Adobe Illustrator

Microsoft PowerPoint, Excel and Word formats are only acceptable if the figure was created in one of these packages.

Do not supply images embedded in Microsoft documents.

Postscript images should be saved in such a way that objects (lines and text) can be selected and edited. If possible, avoid converting text to outline; use the latest version of your software when saving. Postscript files can usually be created using either the "Save as" command or the "Export" command. These can usually be found under the File menu. Alternatively, if you have a postscript printer driver you can select Print from the file menu and then select "Print to file". This will create a postscript file (.ps or .prn), which can then be converted to EPS or PDF.

File compression should not be used.

TeX/LaTeX-coded figures should be converted to postscript format (PS, EPS or PDF).

Figure permissions

Figures from other sources should be fully acknowledged in the caption, and written permission sought for both print and electronic reproduction before being used. It is the author's responsibility to obtain permission to use copyright material.

Colour figures

Colour figures should be supplied in CMYK mode. Converting RGB images to CMYK can affect the appearance of images, which is why we prefer the author to do this before submitting. If you are unsure about how to convert files, please email the [production office](#) with an explanation of how they were created (including the software used), and we will advise.

Owing to the high cost of colour origination and printing, authors are asked to contribute towards the extra costs. Colour costs are as follows:

£300 plus VAT (UK current rate), where applicable, for 1-2 colour figures.

Manuscript Central

To submit a paper to the journal, ensure you have the following files prepared:

- a text file (doc, txt, etc). This should include all text, including figure legends.

- all figures supplied as separate files in EPS format, or the format native to the software in which they are created. Do not include legends, just a simple description, e.g. Figure 1 (a).

If you have any questions regarding file types, please email the [editorial office](#).

Spelling

Spelling should conform to the preferred spelling of the *Shorter Oxford English Dictionary*.

Units and abbreviations

As far as possible the recommendations based on the International System of Units (SI) contained in *Quantities, units and symbols* should be followed (The Royal Society, 1975, price £3.50, available from the Sales Office on +44 (0)20 7451 2645).

Abbreviations should be given in full at the first mention. In the list of references, widely recognized abbreviations for journals should be used. If in doubt, give the full title of the journal.

Data and material sharing, database deposit and Electronic Supplementary Material (ESM)

Data should be made available either through an appropriate, recognized data repository or by utilising the Electronic Supplementary Material (ESM) associated with your paper.

If you choose to use a repository, the associated accession number, link or DOI to the dataset(s) must be included in the methods section of the article. For example:

- Data deposited in the Dryad repository: <http://dx.doi.org/10.5061/dryad.xxxxx>
Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available). For example:

- Author names. Year, Data from: Title of paper. Dryad Digital Respository. <http://dx.doi.org/10.5061/dryad.xxxxx>

Where no discipline-specific data repository exists authors should deposit their datasets in a general repository such as Dryad (<http://datadryad.org/>).

Where possible any other relevant research materials (such as statistical tools, protocols, software etc) should also be made available and details of how they may be obtained should be included in the methods section of the article.

Authors must disclose upon submission of the manuscript any restrictions on the availability of research materials or data.

We can also host electronic supplementary material (ESM) onto our website, which may include, for example, large data sets, movies, figures and tables. However, the main

report, published in the printed journal, should stand on its own merit. Please note that the number of references included in the ESM should be kept to **an absolute minimum** as these are not recognised by many indexing services. Note that ESMs are created by the authors themselves and are **NOT** edited by the Royal Society so please proof-read these thoroughly before submitting and ensure that all references are in the Vancouver format. ESMs will be considered in the following file formats only:

*.AIF, *.AVI, *.CEL, *.CLASS, *.CSS, *.CSV, *.DOC, *.DOCM, *.DOCX, *.DOTM, *.DOTX, *.DTL, *.DVI, *.EPS, *.FAS, *.GIF, *.GZ, *.HTM, *.HTML, *.HTSLP, *.JAR, *.JAVA, *.JPEG, *.JPG, *.JS, *.M, *.M4A, *.MML, *.MOV, *.MP3, *.MP4, *.MPG, *.MPGA, *.NB, *.NET, *.NEX, *.ODE, *.ONEPKG, *.ONETMP, *.ONETOC, *.PBM, *.PDB, *.PDF, *.PGM, *.PNG, *.POTM, *.POTX, *.PPAM, *.PPM, *.PPSM, *.PPSX, *.PPT, *.PPTM, *.PPTX, *.PS, *.PSD, *.RA, *.RM, *.RTF, *.RV, *.SHTML, *.SLDM, *.SLDX, *.SPT, *.SVG, *.SWF, *.TAR, *.TEX, *.TGZ, *.THMX, *.TIF, *.TSV, *.TXT, *.VMD, *.WAV, *.WMA, *.WMV, *.XLAM, *.XLS, *.XLSB, *.XLSM, *.XLSX, *.XLTM, *.XLTX, *.XML, *.XSL, *.XYZ.

Where possible we request that authors combine multiple ESM files into one file (for example, where ESM files are in Word or PDF format).

Authors should submit ESMs as supporting files with their submission through ScholarOne Manuscripts including titles and descriptions in the submission form. There is a size limit of 10MB for ESM (limit for total material, not per file), due to the difficulties that some users will experience in loading or downloading files. Authors with ESM files of a larger size (in particular, movies) should contact the Editorial Team for further assistance.

Publishing Ethics and other policies

This describes the Royal Society's position on the major ethical principles of academic publishing. Authors, editors and referees are asked to comply with this policy.

Preprint

In relation to the **Preprint** version only, the Author is free to post it on web sites, including electronic preprint servers. When the Definitive Published Version of the article is published the Author must acknowledge it by placing the full bibliographic reference and URL of the relevant journal homepage close to the title of the Article.

Language polishing and author resources

Authors who believe their manuscripts would benefit from professional editing prior to submission are encouraged to use a language polishing service.