UNIVERSIDADE ESTADUAL DE CAMPINAS

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DESCONEXÃO DE HÁBITATS
E O DECLÍNIO GLOBAL DOS ANFÍBIOS

Dissertação apresentada ao Instituto de Biologia para obtenção do Título de Mestre em Ecologia.

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Campinas, 2007

FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DO INSTITUTO DE BIOLOGIA – UNICAMP

B388d

Becker, Carlos Guilherme
Desconexão de hábitats e o declínio global dos anfíbios / Carlos Guilherme Becker. – Campinas, SP: [s.n.], 2007.

Orientadores: Paulo Inácio de Knegt López de Prado, Carlos Roberto Sörensen Dutra da Fonseca.
Dissertação (mestrado) – Universidade Estadual de Campinas, Instituto de Biologia.

1. Declínio de anfíbios. 2. Migração reprodutiva. 3. Fragmentação florestal. 4. Matriz de habitat. 5. Macroecologia. I. Prado, Paulo Inácio de Knegt López de. II. Fonseca, Carlos Roberto Sörensen Dutra da. III. Universidade Estadual de Campinas. Instituto de Biologia. IV. Título.

(rcdt/ib)

Título em inglês: Habitat-split and the global decline of amphibians.
Palavras-chave em inglês: Amphibian declines; Breeding migration; Forest fragmentation; Matrix habitat, Macroecology.
Área de concentração: Ecologia.
Titulação: Mestre em Ecologia.
Banca examinadora: Paulo Inácio de Knegt López de Prado, Paula Cabral Eterovick, André Victor Lucci Freitas.
Data da defesa: 23/03/2007.
Programa de Pós-Graduação: Ecologia.

Campinas, 23 de março de 2007

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Dedico este trabalho à Mônica, meu amor...

e à minha família

Agradecimentos

Agradeço primeiramente aos meus pais Evidio Becker e Idalice Becker pelo amor, educação e incentivo. Agradeço também à minha irmã Débora por tudo que tem feito pela família, por mim, e por este trabalho. À minha noiva Mônica por ter estado ao meu lado nos momentos felizes e difíceis durante este mestrado. Sua ajuda tanto em campo quanto em laboratório foi indispensável para que este trabalho tivesse um final feliz; muito obrigado por tudo. Agradeço também à família Kersch por todo o apoio e a confiança depositada em mim.

Agradeço profundamente aos meus grandes amigos e co-autores dos três capítulos desta tese: meu orientador Prof. Paulo Inácio Prado, meu co-orientador Prof. Carlos Roberto Fonseca, e ao colaborador deste projeto Prof. Célio Haddad. Ao meu orientador agradeço por todo o apoio durante este trabalho, ao meu co-orientador pelo incentivo desde o início da minha graduação, e ao Prof. Célio por acreditar e contribuir com este trabalho desde nossa primeira conversa.

Aos amigos do Laboratório de Interação Insetos-Plantas pelas sugestões: Prof. Thomas Lewinsohn, Rafael Loyola, Mário A. Neto, Umberto Kubota “por tantas ajudas estatísticas”, Marina R. Braun, Ricardo Fabiano, Paula Perre e Rosane Picon.

Agradeço a Jean Paul Metzger, Paulo Manzani, João Giovanelli e Arthur Furegati pelos auxílios teóricos e logísticos.

Agradeço à Carina Lima da Silveira por ter cuidado bem de mim e da Mônica durante estes dois anos em Campinas.

Aos amigos que se sujeitaram ao sofrimento no campo: Leandro Tavares, Sebastian Sendoya, Carlos Araújo, Guilherme F. Kersch “Mémi”, Nilson Alves “Jhow”, e meu próprio orientador PI.

Aos membros da pré-banca e banca, respectivamente: Dr. Mário Almeida Neto, Dra. Paula Cabral Eterovick e Dr. André Victor Lucci Freitas que dispuseram parte de seus escassos tempos para lerem e direcionarem esta tese para um patamar muito superior.

Agradeço ao Oraculum por me mandar forças do além: Chris Vogt, Guto Steffen, Lê Pezzuol e Heine Wentz.

À equipe do Projeto SLP, especialmente ao meu grande amigo Rômulo Fernandes Batista, pelo auxílio com todas as questões de Sistema de Informação Geográfica - SIG.

Aos proprietários das terras onde foram realizados os trabalhos de campo, que permitiram a instalação das armadilhas.

Ao pessoal da república em que morei: Flávio, Kubota, Aluana, Danilo, Sebastian, Adriana, Joice, Letícia e Larissa. Agradeço também a todos os novos e velhos amigos que, de alguma forma, me apoiaram nestes últimos dois anos.

Finalmente, agradeço à FAEPex e NEPAM pelo apoio financeiro, e principalmente à FAPESP pela bolsa de estudos “proc. n^{o}. 04/13132-3”.

A natureza é rubra em presas e garras

"Nature red in tooth and claw"

Alfred Lord Tennyson (1850)

In Memoriam

Proceratophrys boiei a lápis preto / por Débora Becker.

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Resumo

O nicho dos anfíbios muda drasticamente ao longo da ontogenia, forçando larvas e pós-metamórficos a ocuparem dois hábitats distintos. Em áreas desmatadas, sítios reprodutivos aquáticos e fragmentos florestais podem ser muito desconectados, isolando o hábitat dos girinos do hábitat dos adultos, em um padrão de fragmentação que chamamos de desconexão de hábitats. Neste estudo, avaliamos os impactos da desconexão de hábitats sobre os anfíbios (i) através de migrações reprodutivas e de padrões de abundância populacional dos anfíbios com larva aquática entre fragmentos com e sem riachos em uma paisagem fragmentada de Mata Atlântica; (ii) através do uso diferencial de hábitat por diferentes guildas nesta mesma paisagem; (iii) por meio de uma análise macroecológica avaliando o efeito da desconexão entre riachos e fragmentos florestais sobre anfíbios de serrapilheira ao longo de 13 pontos na Mata Atlântica. Em nível populacional, detectamos um forte padrão de migração dos anfíbios florestais com desenvolvimento larval aquático entre fragmentos sem riachos e os riachos da matriz de pastagem. Estas espécies foram dramaticamente mais abundantes em fragmentos com riachos do que sem riachos. A estrutura de comunidades variou consistentemente entre hábitats, sendo a guilda das espécies florestais de desenvolvimento larval aquático a mais prejudicada pela desconexão. Tratando-se de grande escala geográfica, a desconexão de hábitat foi o único atributo da paisagem afetando negativamente a riqueza de espécies com desenvolvimento larval aquático, enquanto que a perda de habitat teve influência negativa somente nas espécies com desenvolvimento direto. Estes resultados sugerem que paisagens com altas taxas de desconexão entre fragmentos florestais e sítios reprodutivos têm maior chance de sofrerem declínios populacionais, especialmente de anfíbios associados à floresta e com desenvolvimento larval aquático. Estratégias de conservação em qualquer país devem considerar o fortalecimento das leis, tendo em mente a importância das matas de galeria e a configuração de cada paisagem, minimizando a desconexão entre hábitats florestais e os sítios reprodutivos dos anfíbios.

Abstract

The niche of amphibians changes drastically along the ontogeny, forcing larvae and post-metamorphics to occupy two distinct habitats. In deforested areas, aquatic breeding sites and forest fragments can be far apart, isolating the habitat of tadpoles from the habitat of adults, in a landscape pattern we call habitat-split. In this study, we evaluated the impacts of habitat-split on amphibians (i) through breeding migrations and abundance patterns of species with aquatic larvae between fragments with and without streams in a severely fragmented landscape of Brazilian Atlantic Forest; (ii) by the habitat use of different guilds in this landscape; (iii) through a macroecological analysis, evaluating the effect of habitat-split on litter-amphibians throughout 13 Atlantic Forest sites. At the population level, we detected a migration pattern for the stream-breeding forest amphibians between the fragments without streams and the streams of adjacent grass fields. These species were dramatically more abundant in fragments with streams than in fragments without streams. The community structure varied consistently across habitats, being the guild of forest-associated species with aquatic larvae the most affected by habitat-split. At a larger geographic scale, habitat-split was the only landscape attribute negatively affecting the richness of species with aquatic larval stage, whereas habitat loss had negative influences on direct development species only. These results suggest that landscapes with high mismatches between forest fragments and breeding sites are more prone to experience population declines, especially of forest-associated amphibians with aquatic larval stage. Conservation strategies in any country must regard the law reinforcement, considering the importance of riparian buffers and the configuration of each landscape, minimizing the mismatch between non-reproductive habitats and breeding sites for amphibians.

Introdução geral

Desde o primeiro congresso mundial de herpetologia, realizado no ano de 1989 na Universidade de Kent, em Canterbury - Inglaterra, sabe-se que um grande número de populações de anfíbios está em declínio em diversas partes do mundo (Barinaga 1990). Em 1990, já havia registros de supressão populacional de anuros e salamandras nas Américas do Norte, Central e do Sul, Europa, Ásia, África e Austrália (Blaustein & Wake 1990), sendo que este problema parece ter se intensificado a partir das décadas de 1960 e 1970 (Blaustein & Wake 1990, Balmford *et al.* 2003), mesmo em áreas aparentemente protegidas das influências do homem (Blaustein & Wake 1990).

A busca da compreensão do acentuado declínio de anfíbios no mundo tem gerado uma crescente onda de pesquisas conservacionistas desde o início da última década (Fig. 1). Assim, a comunidade científica vem reportando uma série de potenciais causas para estas extinções e supressões populacionais (Alford & Richards 1999, Young *et al.* 2001, Beebee & Griffiths 2005). No topo das listas existentes sobre a importância relativa dos fatores que explicam o declínio mundial dos anfíbios estão a perda, fragmentação e alteração de habitat (Alford & Richards 1999, Young *et al.* 2001, Beebee & Griffiths 2005, Cushman 2006). Além disto, existe uma série de evidências para os impactos negativos dos contaminantes químicos (Relyea 2005, Boone *et al.* 2004, Hayes *et al.* 2002), para a disseminação de patógenos (Blaustein & Johnson 2003, Daszak *et al.* 2003, Blaustein *et al.* 2005), especialmente do fungo *Batrachochytrium dendrobatidis* (Ron 2005, Carnaval *et al.* 2006), das mudanças climáticas (Pounds *et al.* 1999, Pounds *et al.* 2006), do aumento da exposição à radiação ultravioleta (Anzalone *et al.* 1998, Palen *et al.* 2002), a da introdução de espécies exóticas (Kiesecker & Blaustein 1998, Vredenburg 2004) e, obviamente, das interações entre muitos destes fatores (Kiesecker *et al.* 2001).

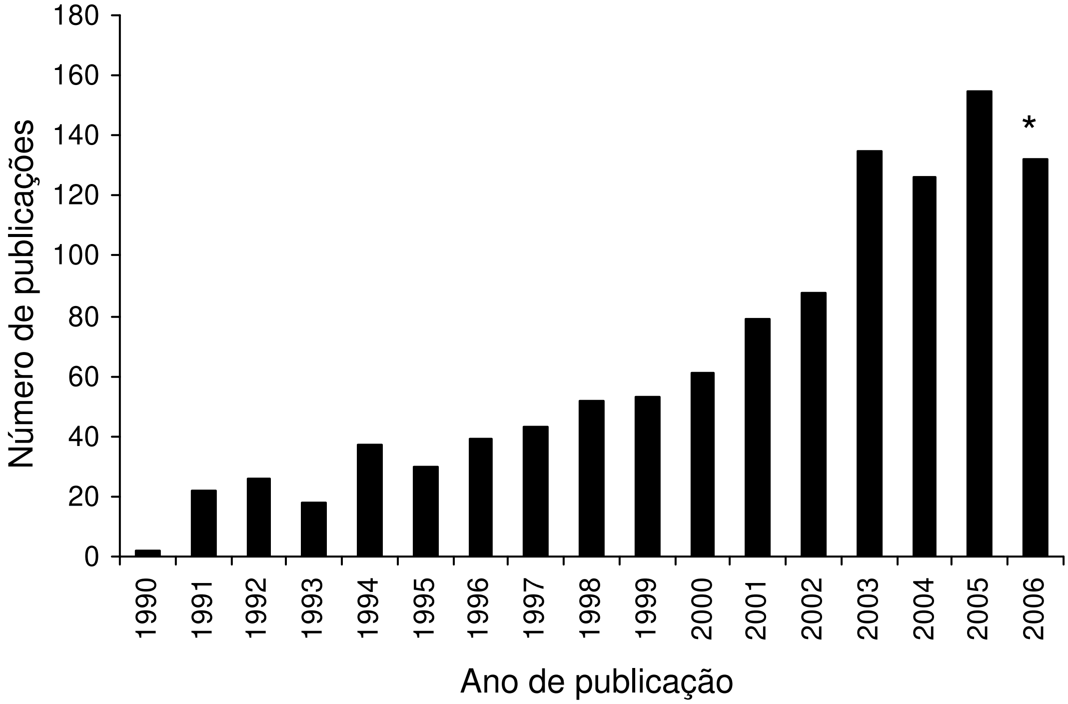


Figura 1 Número de registros de artigos científicos por ano no portal ISI Web of Science utilizando as palavras-chave "Amphibia*" e "declin*". Total de registros no dia 11/01/2007: 1100. * indica banco de dados não finalizado.

Ao se mapear os locais mundiais com os maiores números de anfíbios com registros de supressão populacional, percebe-se que todos apresentam relevo montanhoso e vegetação florestal (Drost & Fellers 1996, Lips *et al.* 2003, Bustamante *et al.* 2005, Eterovick *et al.* 2005, Hero *et al.* 2005). Em muitos destes casos, o uso antrópico da terra é concentrado nas regiões próximas aos cursos d'água, que são em geral mais produtivas e de fácil acesso (Viana *et al.* 1997), fazendo com que a maioria dos fragmentos florestais se concentre nas porções mais elevadas e secas da paisagem, como topos de morro e locais com grande inclinação. Nesta tese propõe-se a hipótese de que este padrão espacial de fragmentação florestal, que leva frequentemente a situações extremas de desconexão entre o habitat florestal dos adultos e o habitat aquático dos girinos, é um importante fator responsável pelo declínio populacional dos anfíbios no mundo. Talvez não seja coincidência que os anfíbios com reprodução associada aos riachos apresentem um número desproporcionalmente grande de registros de declínio em diversos biomas (Lips *et al.* 2003, Hero *et al.* 2005, Bustamante *et al.* 2005).

Anfíbios associados a riachos são de grande importância na dinâmica das teias tróficas e no fluxo de energia dos ambientes aquáticos (Whiles *et al.* 2006), influenciando na produção primária, no ciclo de nutrientes, na decomposição de serrapilheira, e no controle das populações de invertebrados (Flecker *et al.* 1999, Ranvestel *et al.* 2004). Experimentalmente, a exclusão de girinos de dois riachos no Canadá causou um aumento dramático na quantidade de clorofila, comprometendo toda a fauna associada aos riachos (Mallory & Richardson 2005). Além de serem importantes reguladores "top-down" dos ecossistemas aquáticos, os anfíbios contribuem com uma surpreendente porção da biomassa e abundância em sistemas florestais (Stewart & Woolbright 1996) e com o fluxo de energia, já que são os vertebrados que melhor convertem energia em biomassa (Pough 1980). Deste modo, uma eventual extinção em massa de anfíbios certamente teria impactos profundos tanto em ecossistemas aquáticos quanto em ecossistemas terrestres.

No Brasil vivem 776 das aproximadas 6000 espécies de anfíbios do mundo (SBH 2005), Somente na Mata Atlântica, um dos mais ameaçados *hot-spots* de biodiversidade (Myers *et al.* 2000, Tabarelli *et al.* 2005), existem aproximadamente 300 espécies (Haddad 1997). É exatamente neste bioma, caracterizado por possuir uma luxuriante vegetação florestal associada a um relevo particularmente acidentado, que se registra a maioria dos de declínios populacionais de anfíbios para o Brasil, sendo a maior parte de espécies endêmicas e com reprodução associada a riachos (Eterovick *et al.* 2005, Haddad 2005). É neste contexto ecológico que se testa, através de informações de campo e de literatura, a hipótese da desconexão de habitat em nível das populações, da comunidade e do bioma.

Referências

Alford, R. A., & S. J. Richards. 1999. Global amphibian declines: A problem in applied ecology. Annual Review of Ecology and Systematics **30**:133-165.

Anzalone, C. R., L. B. Kats, & M. S. Gordon. 1998. Effects of solar UV-B radiation on embryonic development in *Hyla cadaverina, Hyla regilla*, and *Taricha torosa*. Conservation Biology **12**:646-653.

Balmford, A., R. E. Green, & M. Jenkins. 2003. Measuring the changing state of nature. Trends in Ecology & Evolution **18**:326-330.

Barinaga, M. 1990. Where have all the froggies gone. Science **247**:1033-1034.

Beebee, T. J. C., & R. A. Griffiths. 2005. The amphibian decline crisis: A watershed for conservation biology? Biological Conservation **125**:271-285.

Blaustein. A. R., & D. B. Wake. 1990. Declining amphibian populations – a global phenomenon. Trends in Ecology and Evolution **5**:203-204.

Blaustein, A. R., & P. T. Johnson. 2003. The complexity of deformed amphibians. Frontiers in Ecology and the Environment **1**:87-94.

Blaustein, A. R., J. M. Romansic, E. A. Scheessele, B. A. Han, A. P. Pessier, & J. E. Longcore. 2005. Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batracbochytrium dendrobatidis*. Conservation Biology **19**:1460-1468.

Boone, M. D., R. D. Semlitsch, J. F. Fairchild, & B. B. Rothermel. 2004. Effects of an insecticide on amphibians in large-scale experimental ponds. Ecological Applications **14**:685-691.

Bustamante, M. R., S. R Ron, & L. A. Coloma. 2005. Cambios em la diversidad em siete comunidades de anuros en los Andes de Ecuador. Biotropica **37**:180-189.

Carnaval, A., R. Puschendorf, O. L. Peixoto, V. K. Verdade, & M. T. Rodrigues. 2006. Amphibian chytrid fungus broadly distributed in the Brazilian Atlantic Rain Forest. Ecohealth **3**:41-48.

Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. Biological Conservation **128**:231-240.

Daszak, P., A. A. Cunningham, & A. D. Hyatt. 2003. Infectious disease and amphibian population declines. Diversity and Distributions **9**:141-150.

Drost, C. A., & G. M. Fellers. 1996. Collapse of a regional frog fauna in the Yosemite area of the California Sierra Nevada, USA. Conservation Biology **10**:414-425.

Eterovick, P. C., A. Carnaval, D. M. Borges-Nojosa, D. L. Silvano, M. V. Segalla, & I. Sazima. 2005. Amphibian declines in Brazil: An overview. Biotropica **37**:166-179.

Flecker, A. S., B. P. Feifarek, & B. W. Taylor. 1999. Ecosystem engineering by a tropical tadpole: Density-dependent effects on habitat structure and larval growth rates. Copeia **2**:495-500.

Haddad, C. F. B. 1997. Biodiversidade dos Anfíbios No Estado de São Paulo. Páginas 15-26 em: R. C. M. Castro, C. A. Joly, & C. E. M. Bicudo, editor. Biodiversidade do Estado de São Paulo, Brasil: síntese do conhecimento ao final do século XX. FAPESP, São Paulo.

Haddad, C. F. B. 2005. Anfíbios. Páginas 59-63 em A. B. Machado, C. S. Martins, e G. M. Drummond, editores. Lista da Fauna Brasileira Ameaçada de Extinção. Biodiversitas, Belo Horizonte.

Hayes, T. B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A. A. Stuart, & A. Vonk. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proceedings of the National Academy of Sciences of the United States of America **99**:5476-5480.

Hero, J. M., S. E. Williams, & W. E. Magnusson. 2005. Ecological traits of declining amphibians in upland areas of eastern Australia. Journal of Zoology **267**:221-232.

Kiesecker, J. M., & A. R. Blaustein. 1998. Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth, and survival of native red-legged frogs *Rana aurora*. Conservation Biology **12**:776-787.

Kiesecker, J. M., A. R. Blaustein, & L. K. Belden. 2001. Complex causes of amphibian population declines. Nature **410**:681-684.

Lips, K. R., J. D. Reeve, & L. R. Witters. 2003. Ecological traits predicting amphibian population declines in Central America. Conservation Biology **17**:1078-1088.

Mallory, M. A., & J. S. Richardson. 2005. Complex interactions of light, nutrients and consumer density in a stream periphyton-grazer (tailed frog tadpoles) system. Journal of Animal Ecology **74**:1020-1028.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, & J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature **403**:853-858.

Palen, W. J., D. E. Schindler, M. J. Adams, C. A. Pearl, R. B. Bury, & S. A. Diamonds. 2002. Optical characteristics of natural waters protect amphibians from UV-B in the US Pacific Northwest. Ecology **83**:2951-2957.

Pough, F. H. 1980. Advantages of ectothermy for tetrapods. American Naturalist **115**:92-112.

Pounds, J. A., M. P. L. Fogden, & J. H. Campbell. 1999. Biological response to climate change on a tropical mountain. Nature **398**:611-615.

Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, & B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature **439**:161-167.

Ranvestel, A. W., K. R. Lips, C. M. Pringle, M. R. Whiles, & R. J. Bixby. 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. Freshwater Biology **49**:274-285.

Relyea, R. A. 2005. The lethal impact of roundup on aquatic and terrestrial amphibians. Ecological Applications **15**:1118-1124.

Ron, S. R. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. Biotropica **37**:209-221.

SBH. 2005. Lista de espécies de anfíbios do Brasil. Sociedade Brasileira de Herpetologia (SBH). Disponível em: http://www.sbherpetologia.org.br/ checklist/anfibios.htm, acessado em 11/01/2007.

Stewart, M., and L. Woolbright. 1996. Amphibians. Páginas 274-320 em D. Reagan, & R. Waide, editores. The Food Web of a Tropical Rain Forest. The University of Chicago Press, Chicago.

Tabarelli, M., L. P. Pinto, J. M. C. Silva, M. Hirota, & L. Bede. 2005. Challenges and opportunities for Biodiversity conservation in the Brazilian Atlantic Forest. Conservation Biology **19**:695-700.

Viana, V. M., A. A. J. Tabanez, and J. L. Batista. 1997. Restoration and management of fragmented landscapes. Páginas 347-365 em W. F. Laurance, & R. O. Bierregaard, editores. Tropical Forest Remnants: Ecology, Management and Conservation of Fragmented Communities. University of Chicago Press, Chicago.

Vredenburg, V. T. 2004. Reversing introduced species effects: Experimental removal of introduced fish leads to rapid recovery of a declining frog. Proceedings of the National Academy of Sciences of the United States of America **101**:7646-7650.

Whiles, M. R., K. R. Lips, C. M. Pringle, S. S. Kilham, R. J. Bixby, R. Brenes, S. Connelly, J. C. Colon-Gaud, M. Hunte-Brown, A. D. Huryn, C. Montgomery, & S. Peterson. 2006. The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. Frontiers in Ecology and the Environment **4**:27-34.

Young, B. E., K. R. Lips, J. K. Reaser, R. Ibanez, A. W. Salas, J. R. Cedeno, L. A. Coloma, S. Ron, E. La Marca, J. R. Meyer, A. Munoz, F. Bolanos, G. Chaves, & D. Romo. 2001. Population declines and priorities for amphibian conservation in Latin America. Conservation Biology **15**:1213-1223.

Capítulo I

Habitat-split and the decline of Atlantic Forest stream-amphibian populations

Abstract

The niche of many amphibians changes drastically along the ontogeny, forcing tadpoles and adults to occupy two distinct environments. In deforested areas, streams and forests can be far apart, isolating the habitat of reproduction from the habitat of shelter and feeding, in a landscape pattern we call habitat-split. In this study we assessed the impacts of habitat-split on stream-breeding forest amphibian populations in a severely fragmented landscape of Atlantic Forest by (i) the evaluation of the transit of stream-breeding forest amphibians between the upland forest fragments, without reproductive sites, and the adjacent streams of pasture matrix; (ii) the comparison of abundance of amphibians between forest fragments with streams and forest fragments without streams; (iii) the comparison of abundance and age structure of stream-breeding forest species between forest streams and matrix streams. We detected a movement pattern for the stream-breeding forest amphibians from the fragments without streams to the matrix streams in the beginning of the rainy season and the opposite movement in the end of the reproductive period. The stream-breeding species associated to forest were dramatically more abundant in fragments with streams than in fragments without streams. Nonetheless, we found no differences in the number of captures and age structure for these species between forest streams and matrix streams. Our results suggest that landscapes with high mismatches between forest patches and breeding sites are radically more prone to experience amphibian population declines, especially of forest-associated species with aquatic larval stage. Even with the lower number of captures of stream-breeding forest amphibians in forest fragments without streams in comparison to forest fragments with streams, we did not detect differences in number of captures and in age structure between the breeding sites of matrix and of forest. This suggests high mortality rates of dispersing juveniles, and not the immediate reproductive failure of each species at matrix streams. Conservation strategies in any country must regard the law reinforcement, considering the importance of riparian buffers and the configuration of each landscape, minimizing the mismatch between forest habitats and breeding sites for amphibians.

Introduction

The niche of amphibians changes drastically along the ontogeny, forcing larvae and post-metamorphics to occupy two distinct habitats (Werner & Gilliam, 1984). In pristine environments or large remnants, reproductive adults easily reach the breeding sites while the just-metamorphosed individuals find their adequate habitat a few meters away. In deforested areas, however, streams and forests can be far apart, isolating the habitat of tadpoles from the habitat of the adults, in a landscape pattern we call habitat-split.

Habitat-split can be analogous to landscape complementation (Dunning *et al.* 1992), where individuals must travel between landscape fragments, since the critical resources are found in patches of different types. This pattern is widespread in fragmented landscapes, especially in regions with mountainous or wavy relief, where forest fragments are usually concentrated in less suitable areas for agriculture, such as steeper slopes and hilltops (Viana *et al*. 1997, Silvano *et al.* 2005, Silva *et al.* 2006). In this scenario of mismatched aquatic and terrestrial habitats, forest amphibian species with aquatic larval stage need to face the multiple hazards of reproductive migrations through the hostile open habitats, which usually form the landscape matrix.

At the individual level, transit between forests and reproductive sites through the landscape matrix dispends a great amount of energy and may be hindered by dehydration, predation and constraints of orientation (Dood Jr & Cade 1998, Rothermel 2004, Mazerolle & Desrochers 2005). All these risks are potentially more intense for the just metamorphosed individuals (Rothermel 2004). As noted by Lima & Zollner (1996), habitat fragmentation is an ultra recent occurrence from an evolutionary standpoint, making juvenile forest-associated amphibians become disoriented when they emerge at non-forest breeding sites. Juveniles may indeed have a limited perceptual range that precludes them from detecting forested areas even only a few meters away (Rothermel 2004), and are less inclined to find the adequate habitat successfully (Mazerolle & Desrochers 2005). At the population level, considering the low transit success between the fragment and the isolated reproductive sites, the likely deficient reproduction at matrix, and also the high mortality of juveniles crossing hostile habitats, local extinctions of forest amphibian species are expected.

Breeding migrations of amphibians through the hostile matrix habitats are poorly documented in fragmentation studies. The isolation between forest fragments and the aquatic breeding sites is expected to have higher impacts on forest-associated amphibians with aquatic larval stage than on species with direct development. Perhaps not coincidently, forest amphibians with aquatic larval stage have disproportional higher records of population decline worldwide when compared to species with other reproductive modes (Lips *et al.* 2003, Hero & Morrison 2004, Bustamante *et al.* 2005, Eterovick *et al.* 2005, Hero *et al.* 2005). Furthermore, these records of population declines have been documented essentially in areas most likely to have habitat-split, such as mountainous relief regions of Eastern Australia, Central America, the Tropical Andes, Brazilian Atlantic Forest and mountainous regions of the United States (Blaustein & Wake 1990).

Our goal in this study was (i) to assess the impacts of habitat-split on stream-breeding forest amphibian populations at landscape scale by the evaluation of the transit of stream-breeding forest amphibians between the upland forest fragments without streams, and the adjacent streams in a grass field matrix; (ii) to compare the abundance of amphibians between forest fragments with streams and forest fragments without streams; (iii) to compare the abundance and age structure of stream-breeding forest amphibian species between forest streams and matrix streams.

Methods

Study site

The study area is in the Brazilian Atlantic Forest domain, one of the most threatened biodiversity hot-spots (Myers *et al.* 2000). The high biodiversity of this biome, including about 300 endemic amphibian species (Haddad 1997), has been dramatically endangered by land-use, mostly by agriculture, cattle-raising, and urbanization (Viana *et al.* 1997, Dale & Pearson 1997, Dean 1997, Morellato & Haddad 2000, Tabarelli *et al.* 2005).

Our study area is a forest fragmented landscape in southeastern Brazil, located between the inland Mantiqueira mountain range and the coastal Serra do Mar mountain range (23°13’ S, 45°20’ W). To describe the area and define the studied fragments we used a 2002 land cover classification (5-meter resolution SPOT image), and hydrographic maps (IBGE 1:50.000). The current situation of streams was checked through an exhaustive field search.

The area is representative of the severe fragmentation suffered by the Atlantic Forest and since the 1930’s is dominated by pasture matrix (59.8% of the cover), which occupies the most accessible, irrigated, and most productive areas of the landscape. Forests currently cover only 11.8% of the landscape, scattered in small and disturbed fragments. The other areas of the landscape are covered by early-growth forests (21.4%), *Eucalyptus* monoculture (3.7%), and urban areas (3.3%). The studied landscape covers 24,376 ha, with altitudes ranging from 756 to 1,080 m, and with 69 forest fragments ranging in size from 10 to 252.5 ha, located mainly in higher quotas of the terrain (forest fragments altitudinal mean = 843.54 m ± 7.95 SE, N = 100;

pastures altitudinal mean = 815.81 m ± 7.74 SE, N = 100). Among the forest patches, only three are crossed by a permanent stream. The study area has 11.76% of forest, being that 9.79% of the total length of drainage ditches is in natural forest cover. Excluding the first order drainages of the analysis, which are usually dry even in rainy seasons (personal field observation), just 8.56% of streams flow in the forests. In the studied landscape streams and microhabitats formed by them (e.g., ponds, swamps) are indeed the main breeding resource for the species with aquatic larvae. In the present study the definition of a species as a stream breeder refers to species reproducing in rivulets and assoriated microhabitats.

The mean annual rainfall is 1,277 mm (ANA 2006) with the rainy season beginning in November with a mean rainfall of 112.4 mm. The rainy season reaches the monthly precipitation peak in January (230.4 mm), and finishes in March (155.8 mm). In the rest of the year the rainfall does not exceed 100 mm monthly, with the driest month being August (27.1 mm) (ANA 2006). The mean annual temperature is 20 °C, with the lowest mean in July (12 °C) and highest mean in February (27 °C) (Radambrasil 1983).

Species

Of the 13 species of amphibians recorded in the studied landscape, we reported results of migrations and abundances for the four species abundant in forests and with aquatic larvae. The two common toads recorded (*Chaunus ictericus* and *Chaunus ornatus;* Bufonidae) are widespread in the Atlantic Forest, inhabiting elevated forests, but are also found in grassland. During breeding time they are found in streams and standing water bodies, such as lakes, ponds or puddles (Kwet & Di-Bernardo 1999, Baldissera *et al.* 2004). The Debigotes frog (*Leptodactylus mystacinus*; Leptodactylidae) is widespread in the Atlantic Forest domain, being found in primary and secondary forests and also in disturbed areas. Although this species has been found in forest in the present study, in other places it is considered a species of open formations, being found also in Cerrado and Chaco. This species can breed in a wide variety of wetlands, such as streams, lakes, and temporary ponds, and the reproduction occurs in excavated clumbers where the eggs are laid in foam nests under the ground with the larvae developing in water (Heyer *et al.* 2003). The horned frog (*Proceratophrys boiei*; Cycloramphidae) is widespread in the Atlantic Forest. This species inhabits primary and secondary forests and preferably breeds in rocky streams or rocky pools (Haddad & Sazima 1992). In the study site, the breeding periods for these four species overlap with the rainy season.

The other four forest-associated species (*Eleutherodactylus binotatus, Eleutherodactylus guentheri, Eleutherodactylus parvus,* and *Leptodactylus marmoratus*) have direct development of tadpoles or endotrophic tadpole development, using the leaf litter as nesting sites. The remaining five species are associated to open habitats (*Elachistocleis ovalis, Leptodactylus furnarius, Leptodactylus labyrinthicus, Physalaemus cuvieri,* and the exotic species *Lythobates catesbeianus*), being dependent on water to reproduce.

Sampling design

We structured the sampling design comparing three forest fragments (> 10 ha) without streams (12.53, 17.42 and 42.34 ha) with the only three available forest fragments (> 10 ha) with streams in the studied landscape (19.27, 20.30, and 126.93 ha) (Fig. 1A). At the fragments without streams we sampled three habitats: upland forest in the fragment (forest - F); the closest stream in the adjacent pasture matrix (matrix stream - MS), and the adjacent pasture matrix between the upland fragment and the matrix stream (matrix - M) (Fig. 1B). In the fragments with streams we sampled in the upland forest (forest - F), and in the stream inside the forest (forest stream - FS) (Fig 1C). The forest streams were all small second or first order creeks in steep drainages, 0.45 – 2.00 m wide and 10 – 50 cm deep. In the pasture matrix the sampled streams were all of second order, larger, (0.45 – 2.50 m wide and 15 – 50 cm deep) and in sanded flat valleys.

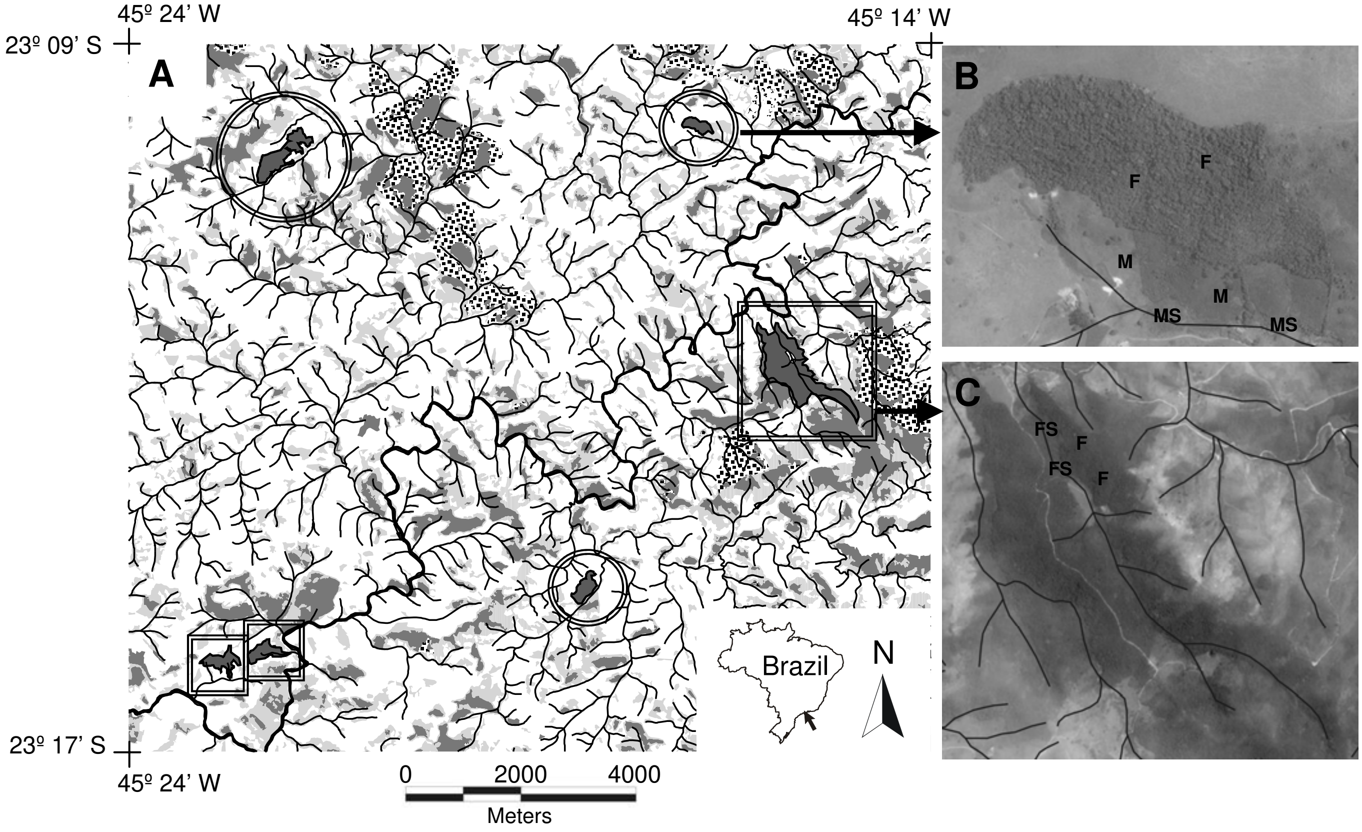


Figure 1 (A) The location of the six studied forest fragments in a wavy relief landscape in Brazilian Atlantic Forest; the circles show the fragments without streams; the squares show the fragments with streams. (/\/) drainages; ☐ , grass field matrix; ▨ , *Eucalyptus* monoculture; ░ , second growth forest; ▓ , forest. (B) Scheme of one of the three forest fragments without stream with traps location in the three habitat categories: F, forest; M, adjacent matrix; MS, adjacent matrix stream. (C) Scheme of one of the three forest fragments with stream with traps location in the two habitat categories: F, forest; FS, forest stream.

We sampled amphibians using pit-fall traps with drift fences adapted from Halliday (1996). Each trap consisted of a 45 m long and 0.6 m tall plastic fence supported by wooden stakes, intercepting four equidistant 60 l buried plastic buckets. To register the individual movement directions through the pastures we glued a plastic partition inside each bucket in the matrix habitats, which thus were divided in a half facing the forest patch, and the other facing the stream in the pasture. All the traps in the matrix were surrounded by wire fences 1.70 m high to avoid damages by cattle or horses.

To determine the position of the traps in forest patches we designed places randomly, but respected the following criteria: (i) the traps should be at least 30 m away from the fragment edge, (ii) they should be 70 m apart from each other, and (iii) all traps should be oriented by contour lines. The upland forest traps of the fragments with streams were installed in higher portions of the relief, at least 100 m from the forest stream.

For the installation of traps near the streams we randomized two points along the streambed and followed the criteria of: (i) each trap should be 70 m apart from the other stream trap; (ii) stream traps should be at maximum distance of 4 m from the streambed and with drift fence oriented parallel to the streambed; (iii) the forest stream traps (FS) should be at least 30 m from the fragment edge; the matrix stream traps (MS) were installed in the fragment side of the stream.

The matrix traps (between fragments and matrix streams - M) were oriented parallel to the fragment edge and matrix stream.

The sampling design comprehends three pairs of traps for each habitat category both in the forest fragments without streams (F, M, MS), and in forest fragments with streams (F, FS), totaling 30 traps. The traps remained open for 10 days.month during five months (November

2004 to March 2005), totaling an effort of 1500 traps.day. To access the precipitation during the study period we used a plastic pluviometer placed on the center of the landscape (23°13’ S, 45°20’ W).

For each trapped individual we identified the species and sex, and for the matrix traps we registered the side of the division in the bucket in which it was captured (upland forest or matrix stream side). Also, each captured individual was weighed with portable scales to the nearest 0.1 g, marked, and released five meters away from the trap, at the other side of the trap. The individual marking was made by single toe-clipping, which registered the census and habitat (forest or matrix) (adapted from Halliday 1996). No recaptures among censuses or traps were recorded and for the very few individuals recaptured at the same trap and on the same census we used only their initial capture data in the analysis.

Statistical analysis

We used data of all matrix (M) and matrix stream traps (MS) for the migration analysis. We tested movement direction biases by comparing the number of captures between the two partitions of the bucket traps using Goodness-of-fit test (G) (Sokal & Rohlf 2000).

We performed nested ANOVAs to compare population abundances between fragments with streams (F traps) and fragments without streams (F traps), and between forest streams (FS traps) and matrix streams (MS traps). For both comparisons we had the contrasting treatments (F x F, and FS x MS) in distinct forest fragments (with streams and without streams), which configures a nested design (Zar 1999), where fragments are a random factor nested within the treatments. Thus, to test the effects of the treatments we used the variation among patches within each treatment to calculate the mean square error in F-tests. We also used Kruskal-Wallis one-way analysis of variance (Sokal & Rohlf 2000) to compare median body mass between forest streams (FS traps) and matrix streams (MS traps). These analyses were run in the SYSTAT 10.2 package (SYSTAT 2002).

Results

We detected a movement pattern for the stream-breeding forest amphibians from the fragments without streams to the matrix streams in the beginning of the rainy season and the opposite movement in the end of the reproductive period (Fig. 2A). In the beginning of the rainy season, captures for pooled species in the matrix were significantly higher in the bucket divisions on the side of the forest fragments ($G_{Nov} = 12.56$, $df = 1$, $p < 0.001$; $G_{\mathrm{Dec}} = 1.70$, $df = 1$, $p < 0.05$), suggesting that the predominant movement across the matrix was from the fragments without streams to the streams in the pasture valleys. In January, there was equal catchability in the two sides of the traps ($G_{Jan} = 0.43$, $df = 1$, $p > 0.5$), and in the end of reproductive period (February and March), the trend was reversed, and captures in the matrix were concentrated in the division of the bucket turned to the pasture streams ($G_{Feb} = 10.63$, $df = 1$, $p < 0.005$; $G_{Mar} = 3.96$, $df = 1$, $p < 0.05$) (Fig. 2B). The pattern was the same excluding the data of the matrix stream traps (MS).

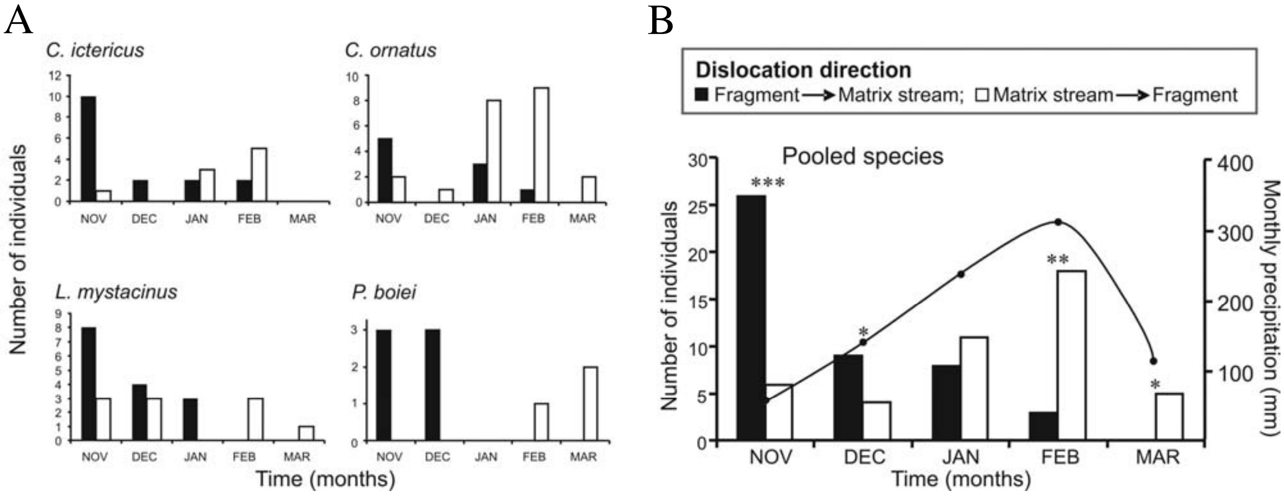


Figure 2 (A) Migration pattern of each stream-breeding species abundant in forest fragments. (B) Migration pattern of four pooled species. Black bars represent upland forest fragments toward matrix streams; white bars represent matrix stream toward upland forest fragment. The line represents the monthly precipitation during the sampling period. Values of *G*: * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.001$.

The stream-breeding species associated to forest were dramatically more abundant in fragments with streams than in fragments without streams (Fig. 3). Fragments without streams had half of captures of *Chaunus ornatus* in average, compared to fragments with streams ($F_{4,7}$ = 9.72, p = 0.036) (Fig. 3A). *Leptodactylus mystacinus* was also less sampled in fragments without streams, with sample size six times smaller ($F_{4,7}$ = 7.81, p = 0.049) (Fig. 3B). For *Proceratophrys boiei* no capture was recorded in dry forest fragments ($F_{4,7}$ = 9.14, p = 0.039) (Fig. 3C). *Chaunus ictericus* was excluded from these analyses due to its small sample size. The difference in number of captures between fragments with and without streams holds even adding the captures of their corresponding streams together (Pooled species; $F_{1,4}$ = 111.364, p < 0.001). Therefore, the differences cannot be attributed purely to the emigration from the fragments without streams during the breeding season.

Nonetheless, we found no differences in the number of captures for these four species between forest streams and matrix streams ($F_{4,7} = 2.332$, $p = 0.201$; $F_{4,7} = 1.25$, $p = 0.327$; $F_{4,7} = 0.385$, $p = 0.569$; $F_{4,7} = 1.350$, $p = 0.310$). Furthermore, no age structure differences were observed between these two stream habitats, only marginally for *Leptodactylus mystacinus* with smaller individuals in forest streams (Kruscal-Wallis test for median body weight differences: *C. ornatus* $U_{1,\ 67} = 468.000$, $p = 0.717$; *L. mystacinus* $U_{1,\ 24} = 44.500$, $p = 0.051$; *P. boiei* $U_{1,\ 31} = 64.000$, $p = 0.234$; *C. ictericus* $U_{1,\ 18} = 53.000$, $p = 0.397$).

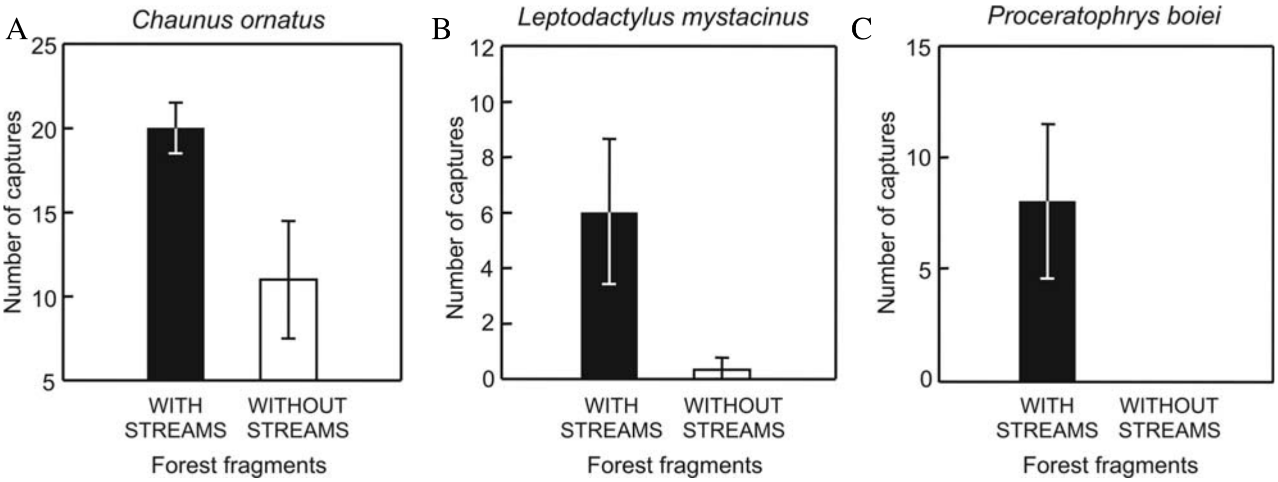


Figure 3 Mean number of captures in forest fragments with streams, black bars; and forest fragments without streams, white bars. Data from forest traps (F). Vertical lines represent ± 1 (SE).

Discussion

Habitat loss and fragmentation are the main causes of species extinction worldwide (Wilcox & Murphy 1985). The mechanisms immediately associated to populations decline are those discussed in the theory of island biogeography (MacArthur & Wilson 1967) and metapopulation models (Hanski & Gilpin 1991, Hanski 1998), such as isolation, inbreeding, and habitat quality. For amphibians, habitat loss and fragmentation have also been indicated as a important cause for global declines (Young *et al.* 2001, Cushman 2006). However, we suggest that habitat-split can be the most important mechanism associated to this biodiversity crisis.

Geographically, habitat-split can occur everywhere. However, it can be expected to be intensified in mountainous landscapes where land use tend to generate non-random fragmentation patterns, where small forest fragments remain on the steeper slopes and hilltops, while the wetter and more accessible lowlands are used for agriculturally productive purposes (Viana *et al.* 1997, Silva *et al.* 2006). This could be one of the reasons why most recorded population declines and extinctions of forest amphibians have occurred in regions with wavy relief such as the east coast of Australia (Hero *et al.* 2005), the Brazilian Atlantic Forest (Eterovick *et al.* 2005), the Andes of South and Central Americas (Young *et al.* 2001, Lips *et al.* 2003, Bustamante *et al.* 2005), and in mountain ranges of the United States (Drost & Fellers 1996).

In terms of life history, stream-breeding forest amphibians could be expected to be more affected by habitat-split than species showing direct development, due to migrations through hostile habitats. Indeed, stream-breeding forest species have a disproportional higher number of threatened species if compared to direct development species worldwide (Lips *et al.* 2003, Hero & Morrison 2004, Hero *et al.* 2005, Bustamante *et al.* 2005, Eterovick *et al.* 2005). In eastern Australia, 20 out of 40 stream-breeding forest species have decline records, contrasting with only one record out of 20 direct development species (Hero *et al.* 2005). In Central America, populations of most riparian species were predicted to decline, as well as some pond breeders and terrestrial species with aquatic reproductive modes (Lips *et al.* 2003). Only those that were exclusively terrestrial and a few species that breed primarily in pools or puddles survived (Lips *et al.* 2003). Bustamante *et al.* (2005) found that only half of the 34 species that have aquatic larvae persisted in seven different sites across the Andes, while all 28 species that have direct development and lack aquatic larvae endured. According to Eterovick et al. (2005), at least 20 Brazilian anuran species deserve attention as to population declines, 18 of those species being related to the Atlantic Forest, and 18 with reproduction associated to streams.

Our results show that landscapes with high mismatches between forest fragments and breeding sites are radically more prone to experience amphibian population declines of forest-associated species with aquatic larval stage. In our study, all stream-breeding species associated to forest were forced to reproduce in presumable sub-optimal conditions of matrix streams. In forest fragments without aquatic breeding sites, where breeding migrations through the matrix are crucial to the maintenance of the population viability, the number of captures was dramatically lower for stream-breeding species. Compared with similar-sized homeotherms, amphibians have higher population densities (Pough 1980), which could allow the maintenance of viable populations in very small forest patches (McGarigal & Cushman 2002). However, the smaller the forest fragment, the smaller are the chances of finding adequate aquatic breeding sites on the forest. In this case, the chances of compulsory migrations through the matrix followed by reproductive failures are higher.

Habitat-split impacts

Forest amphibian species have different responses to landscape matrix (Gascon *et al.* 1999, Tocher *et al.* 2001, Urbina-Cardona *et al.* 2006). Even species which usually avoid open habitats could be forced to cross through the matrix in the absence of breeding sites in the forest. For instance, in a radio-telemetry study conducted by Rittenhouse & Semlitsch (2006) none of the radio-tagged adults of the spotted salamander (*Ambystoma maculatum*) were observed more than a few meters into the grassland, suggesting avoidance of open formations when breeding sites are available in the forest (e.g., pool). In our case, the four stream-breeding forest species where captured in the pasture matrix, mainly close to the streams, showing that they can leave the forest to reproduce in the presumably impacted streams of the matrix.

The stream-breeding species we recorded have a clear seasonal migration pattern through the matrix, and face different adversities along the rainy season. In the beginning of the rainy season, mostly the reproductive adults leave the upland forest fragments downward to streams of the matrix. The most probable adversities faced in this period are dehydration and predation, since adults have higher sense of orientation and the direction is immediately facilitated by the vocalizations of conspecifics in matrix streams and the topographic features. The problems faced in the end of reproductive period are apparently very severe, when not only the adults, but mostly the slow moving juveniles leave the matrix streams to reach the upland forest fragments. Additionally, there is no more vocalization to guide the returning adults. Because of the lower locomotory performance of juveniles (Rogowitz *et al.* 1999, Preest & Pough 2003), the terrain slope may make it difficult for frogs to reach the forests. The end of reproductive period for these stream-breeding forest species corresponds to the end of rainfall, when individuals must reach the forest as fast as possible. Late migrations may decrease the recruitment rates, as the matrix air temperature is still high during the day and climate conditions become more hostile throughout the dry southern hemisphere winter.

Juveniles leaving the streams for the first time probably respond similarly to physical cues in the immediate terrestrial environment, such as moisture gradients or topographic features. Rothermel (2004) found in an experimental study that, in the end of the reproductive period, individuals of the salamander *Ambystoma maculatum* that breed in ponds far away from the forest were likely to return to the pond after a bad search for forests. Rittenhouse & Semlitsch (2006) also found that juveniles initially migrating from the grassland side of a pond reversed their walking direction more often than juveniles that initially emigrated from the forest side of the pond. Furthermore, in an experimental investigation of landscape resistance of forest versus old-field habitats for emigrating amphibians, Rothermel & Semlitsch (2002) found that juveniles of the American toad (*Bufo americanus*) moved more than three times as often to the forest than to the field. In our study, in the end of the breeding period the individuals which moved from the forest to the matrix stream were mostly juveniles, indicating also many reversals to the moisture of the streams. High number of reversals suggests a high number of failures to locate suitable habitats (Rothermel 2004), and also suggests higher risks for juveniles on dispersion and an increasing reproductive failure analogous to habitat-split. Moreover, amphibians apparently do not choose to migrate through forest corridors between non-reproductive habitats and breeding sites (Dodd & Cade 1998, Jenkins *et al.* 2006), causing the effects of habitat-split to be more dramatic.

In an experimental study, Rothermel (2004) found that only 9% of juvenile *Ambystoma maculatum* apparently survived their initial migration into the terrestrial environment. Since dehydration directly suppresses the physiological mechanisms of movement (Gatten 1987, Hillman 1987, Preest & Pough 1989), and has been directly observed to have a negative impact over locomotory performance (Rogowitz *et al.* 1999, Preest & Pough 2003), juveniles suffer much more than adults when crossing hostile habitats. They may also respond differently to non-forested formations than adults, which are familiar with the landscape and experience lower rates of evaporative water loss because of their larger body size (Spight 1968). In our field study we found individuals of *Proceratophrys boiei* and *Chaunus ornatus* dead on pastures in the end of the reproductive period, and without predation evidence, suggesting dehydration as the probable cause of death. A better picture of this mortality could be achieved by a systematic survey of dead anurans in pastures between streams in the valleys and forest patches in he hills.

Due to the high mortality rates in crossing matrixes, the success of recruitment decreases with the distance increasing between the reproductive sites and the forest fragments (Rothermel 2004). Amphibian species with large body size and high dislocation rates surprisingly seem to be more threatened than relatively small and sedentary species (Lips *et al.* 2003). Large species have greater chances of crossing hostile habitats to reproduce in breeding sites disconnected and far away from the upland forest habitats, offering lower survival chances to juveniles as they must cross great distances through hostile habitats. In addition, as the population density may be important influencing population survival, large species are generally more threatened, maybe because of their lower population densities (Lips *et al.* 2003).

The reproductive success of amphibians with aquatic larval stage is strongly affected by sediments (Welsh & Ollivier 1998, Lowe & Bolger 2002), agrochemicals (Hayes *et al.* 2002, Boone *et al.* 2004, Relyea 2005), the presence of exotic species (Kiesecker & Blaustein 1998, Lowe & Bolger 2002, Vredenburg 2004, Brown *et al.* 2006), and the immediate vegetation surrounding their breeding sites (Lowe & Bolger 2002, Brown *et al.* 2006). Success of the reproducing amphibians is also positively associated with conditions experienced by their aquatic larvae (Semlitsch *et al.* 1988). Goater (1994) and Goater & Vandenbos (1997) showed that conditions faced by larvae continued to affect survival immediately after metamorphosis in European toads (*Bufo bufo*) and wood frogs (*Lithobates sylvaticus*), respectively. As well as this, the lower survival was associated with a later metamorphosis for brown frogs *Rana arvalis* and *R. temporaria* (Lyapkov *et al.* 2000, Chelgren *et al.* 2006).

A reduction in terrestrial refuge availability could also increase adult and larval mortality because of natural disturbances caused by floods (Hart & Finelli 1999). For the Brazilian Atlantic Forest, even patchier forests associated with fewer rainy days, there is no affect on the pooled annual rainfall (Webb *et al.* 2005). This indicates that the streams of fragmented forests are more prone to high flow events. Reduction in terrestrial refuge availability could also increase predation rates in matrix streams where the Bullfrog, *Lythobates catesbeianus*, are present. The presence of Bullfrogs in the matrix, which are predators of tadpoles and adults, resource competitors (Kupferberg 1997, Kiesecker & Blaustein 1998), and potential disseminators of diseases like that produced by the amphibian-specific aquatic fungus *Batrachochytrium dendrobatidis* (Daszak *et al.* 2004), may have severe impacts on the recruitment of all stream-breeding species (Carnaval *et al.* 2006). Kupferberg (1997) found that Bullfrog tadpoles affected benthic algae significantly, and then caused a 48% reduction in survival rates of the *R. boylii* and a decline of 24% in their body mass during metamorphosis. Hanselmann *et al.* (2004) found that 96% of the Bullfrogs from the Venezuelan Andes have been contaminated with chytrid fungus *(Batrachochytrium dendrobatidis)* and did not develop the disease, suggesting that Bullfrogs (*L. catesbeianus*) may be an important reservoir for this pathogen. Even with low number of captures in our study, Bullfrogs were recorded widely in grass field matrix and all matrix streams.

Habitat-split and global amphibian declines

Since the last decade the scientific community has been reporting a series of probable causes for amphibian population declines (Alford & Richards 1999, Young *et al.* 2001, Beebee & Griffiths 2005). In relatively undisturbed regions, several cases have been attributed to climate changes (Pounds *et al.* 1999), chytridiomycosis (Berger *et al.* 1998, Lips 1998), and the interaction between both factors (Pounds *et al.* 2006). Nevertheless, we believe that besides habitat loss (Cushman 2006), the high mismatch between forest remnants and aquatic habitats amplifies dramatically the impacts of deforestation in populations of stream-breeding forest amphibians in many regions of the world.

As the original forest habitat has been increasingly lost and fragmented, the chances of available breeding sites being connected to the forest fragments are scarce. Increasing the habitat-split causes compulsory seasonal movements of amphibians between the aquatic and the terrestrial habitats, and also increases the exposure of individuals to multiple hazards. For the forest-associated amphibians with aquatic larvae, upland terrestrial habitats are required during the non-breeding times of the year. When matrix streams are the only choice to reproduce, there are greater chances of individuals being exposed to suboptimal or dangerous conditions such as breeding sites with lower pH (Räsänen *et al.* 2003), higher UV-B radiation (Anzalone *et al.* 1998, Palen *et al.* 2002), higher water temperature, and the presence of agrochemical contaminants (Hayes *et al.* 2002, Boone *et al.* 2004, Relyea 2005). This fragmentation pattern facilitates the invasion of exotic species, and the infestation of parasites and pathogens via matrix vectors (Daszak *et al.* 2003, Daszak *et al.* 2004).

Furthermore, climate shifts are more likely to occur in fragmented forests. For instance, the patchier landscapes of Atlantic Forest are associated with fewer rainy days and higher interannual variability in rainfall (Webb *et al.* 2005). These are unquestionably negative impacts on amphibians' migration, reproduction and on the dynamics of their breeding sites. According to literature, all these factors mentioned above are the main contributors for amphibian population declines (Alford & Richards 1999, Young *et al.* 2001, Beebee & Griffiths 2005), but all these factors are dramatically more intense on landscapes with high habitat-split.

Conservation implications

Even with the lower number of captures of stream-breeding forest amphibians in forest fragments without streams along the rainy season, we did not detect any difference in the number of captures and age structure between the breeding sites (streams) of matrix and forest. This suggests high mortality rates of dispersing juveniles, and not the immediate reproductive failure of each species at matrix streams. Nonetheless, the studied landscape is deeply fragmented and probably experienced a series of extinctions of forest-associated amphibians with aquatic larvae during the last century of massive deforestation. The four stream-breeding forest species we have studied are very common species and have high geographic ranges. They certainly are a small fraction of the original community that endures even with the multiple hazards of reproduction in the impacted breeding sites.

Conversion of forest into pasture, frequently subsidized by governmental incentives, has been one of the leading forces of forest loss and fragmentation in tropical countries (Moran 1993). At least in Brazil, there are presently legal actions that attenuate the habitat-split by protecting the legal reserve of 30-m riparian vegetation surrounding streams and rivers. However, the laws are still not respected. In a multidisciplinary study by Silvano *et al.* (2005) in the Atlantic Rainforest, farmers recognize the usefulness of forests they already have (on hilltops), but at the same time do not want to have more forests inside their properties (on lowlands), because they do not see direct benefits from them, as forested land reduces the available area for pasture. The legal requirement of forest cover in riparian zones and the concern of land owners with the water quality synergizes a good opportunity to recover an adequate scenario for amphibians in fragmented landscapes.

Conservation strategies in any country must regard the law reinforcement, considering the importance of riparian buffers and the configuration of each landscape, minimizing the mismatch between non-reproductive habitats and breeding sites for amphibians. Conservation efforts related to global amphibian declines will not be complete without considering the habitat-split.

References

Alford, R. A., and S. J. Richards. 1999. Global amphibian declines: A problem in applied ecology. Annual Review of Ecology and Systematics **30**:133-165.

ANA, Agência Nacional de Águas. 2006. Pluviosity database. Agência Nacional de Águas, Brasília, Brasil.

Anzalone, C. R., L. B. Kats, and M. S. Gordon. 1998. Effects of solar UV-B radiation on embryonic development in *Hyla cadaverina, Hyla regilla*, and *Taricha torosa*. Conservation Biology **12**:646-653.

Baldissera, F. A., U. Caramaschi, and C. F. B. Haddad. 2004. Review of the *Bufo crucifer* species group, with descriptions of two new related species (Amphibia, Anura, Bufonidae). Arquivos do Museu Nacional (Rio de Janeiro) **62**:255-282.

Beebee, T. J. C., and R. A. Griffiths. 2005. The amphibian decline crisis: A watershed for conservation biology? Biological Conservation **125**:271-285.

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Marantelli, and

H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences of the United States of America **95**:9031-9036.

Blaustein. A. R., and D. B. Wake. 1990. Declining amphibian populations – a global phenomenon. Trends in Ecology and Evolution **5**:203-204.

Boone, M. D., R. D. Semlitsch, J. F. Fairchild, and B. B. Rothermel. 2004. Effects of an insecticide on amphibians in large-scale experimental ponds. Ecological Applications **14**:685-691.

Brown, C. J., B. Blossey, J. C. Maerz, and S. J. Joule. 2006. Invasive plant and experimental venue affect tadpole performance. Biological Invasions **8**:327-338.

Bustamante, M. R., S. R Ron, and L. A. Coloma. 2005. Cambios em la diversidad em siete comunidades de anuros en los Andes de Ecuador. Biotropica **37**:180-189.

Carnaval, A., R. Puschendorf, O. L. Peixoto, V. K. Verdade, and M. T. Rodrigues. 2006. Amphibian chytrid fungus broadly distributed in the Brazilian Atlantic Rain Forest. Ecohealth **3**:41-48.

Chelgren, N. D., D. K. Rosenberg, S. S. Heppell, and A. I. Gitelman. 2006. Carryover aquatic effects on survival of metamorphic frogs during pond emigration. Ecological Applications **16**:250-261.

Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. Biological Conservation **128**:231-240.

Dale, V. H., and S. M. Pearson. 1997. Quantifying habitat fragmentation due to land-use change in Amazonia. Pages 400–410 in W.F. Laurance, and R.O. Bierregaard, editors. Tropical Forest Remnants: Ecology, Management and Conservation of Fragmented Communities.

University of Chicago Press, Chicago.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2003. Infectious disease and amphibian population declines. Diversity and Distributions **9**:141-150.

Daszak, P., A. Strieby, A. A. Cunningham, J. E. Longcore, C. C. Brown, and D. Porter. 2004. Experimental evidence that the bullfrog *Rana catesbeiana* is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herpetological Journal **14**:201-207.

Dean, W. 1997. A ferro e fogo: a história e a devastação da Mata Atlântica brasileira. Companhia das Letras, São Paulo.

Dodd, C. K., and B. S. Cade. 1998. Movement patterns and the conservation of amphibians breeding in small, temporary wetlands. Conservation Biology **12**:331-339.

Drost, C. A., and G. M. Fellers. 1996. Collapse of a regional frog fauna in the Yosemite area of the California Sierra Nevada, USA. Conservation Biology **10**:414-425.

Dunning, J. B., B. J. Danielson, and H. R. Pulliam. 1992. Ecological processes that affect populations in complex landscapes. Oikos **65**:169-175.

Eterovick, P. C., A. Carnaval, D. M. Borges-Nojosa, D. L. Silvano, M. V. Segalla, and I. Sazima. 2005. Amphibian declines in Brazil: An overview. Biotropica **37**:166-179.

Gascon, C., T. E. Lovejoy, R. O. Bierregaard, J. R. Malcolm, P. C. Stouffer, H. L. Vasconcelos, W. F. Laurance, B. Zimmerman, M. Tocher, and S. Borges. 1999. Matrix habitat and species richness in tropical forest remnants. Biological Conservation **91**:223-229.

Gatten, R. E. 1987. Activity metabolism of anuran amphibians - tolerance to dehydration. Physiological Zoology **60**:576-585.

Goater, C. P. 1994. Growth and Survival of Postmetamorphic Toads - Interactions among Larval

History, Density, and Parasitism. Ecology **75**:2264-2274.

Goater, C. P., and R. E. Vandenbos. 1997. Effects of larval history and lungworm infection on the growth and survival of juvenile wood frogs *Rana sylvatica*. Herpetologica **53**:331-338.

Haddad, C. F. B., and I. Sazima. 1992. Anfíbios anuros da Serra do Japi. História Natural da Serra do Japi: Ecologia e Preservação de uma Área Florestal no Sudeste do Brasil. In: L. P. C. Morellato, editor. Editora UNICAMP/FAPESP, Campinas.

Haddad, C. F. B. 1997. Biodiversidade dos Anfíbios No Estado de São Paulo. Pages 15-26 in R. C. M. Castro, C. A. Joly, and C. E. M. Bicudo, editors. Biodiversidade do Estado de São Paulo, Brasil: Síntese do Conhecimento ao Final do Século XX. FAPESP, São Paulo.

Halliday, T. R. 1996. Amphibians. Pages 205-216 in W. J. Sutherland, editor. Ecological Census Techniques: a Handbook. Cambridge University Press, Cambridge.

Hanselmann, R., A. Rodriguez, M. Lampo, L. Fajardo-Ramos, A. A. Aguirre, A. M. Kilpatrick, J. P. Rodriguez, and P. Daszak. 2004. Presence of an emerging pathogen of amphibians in introduced bullfrogs *Rana catesbeiana* in Venezuela. Biological Conservation **120**:115-119

Hanski I. 1998. Metapopulation dynamics. Nature **396**:41-49

Hanski I., and M. Gilpin. 1991. Metapopulation dynamics: Brief history and conceptual domains. Biological Journal of Linnean Society **42**:3-16.

Hart, D. D., and C. M. Finelli. 1999. Physical-biological coupling in streams: the pervasive effects of flow on benthic organisms. Annual Review of Ecology and Systematics **30**:363-395

Hayes, T. B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A. A. Stuart, and A. Vonk. 2002.

Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proceedings of the National Academy of Sciences of the United States of America **99**:5476-5480.

Hero, J. M., S. E. Williams, and W. E. Magnusson. 2005. Ecological traits of declining amphibians in upland areas of eastern Australia. Journal of Zoology **267**:221-232.

Hero, J. M., and C. Morrison. 2004. Frog declines in Australia: global implications. The Herpetological Journal **14**:175-186.

Heyer, M. M., W. R. Heyer, S. Spear, and R. O. de Sá. 2003. *Leptodactylys mystacinus*. Catalogue of American Amphibians and Reptiles **767**:1-11.

Hillman, S. S. 1987. Dehydrational effects on cardiovascular and metabolic capacity in 2 amphibians. Physiological Zoology **60**:608-613.

Jenkins, C. L., K. McGarigal, and B. C. Timm. 2006. Orientation of movements and habitat selection in a spatially structured population of marbled salamanders *Ambystoma opacum*. Journal of Herpetology **40**:240-248.

Kiesecker, J. M., and A. R. Blaustein. 1998. Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth, and survival of native red-legged frogs *Rana aurora*. Conservation Biology **12**:776-787.

Kupferberg, S. J. 1997. Bullfrog *Rana catesbeiana* invasion of a California river: The role of larval competition. Ecology **78**:1736-1751.

Kwet, A., and M. Di-Bernardo. 1999. Pró-Mata – Anfíbios, Amphibien, Amphibians. EDIPUCRS, Porto Alegre.

Lima, S. L., and P. A. Zollner. 1996. Towards a behavioral ecology of ecological landscapes.

Trends in Ecology & Evolution **11**:131-135.

Lips, K. R. 1998. Decline of a tropical montane amphibian fauna. Conservation Biology **12**:106-117.

Lips, K. R., J. D. Reeve, and L. R. Witters. 2003. Ecological traits predicting amphibian population declines in Central America. Conservation Biology **17**:1078-1088.

Lowe, W. H., and D. T. Bolger. 2002. Local and landscape-scale predictors of salamander abundance in New Hampshire headwater streams. Conservation Biology **16**:183-193.

Lyapkov, S. M., V. G. Cherdantsev, E. M. Cherdantseva, and A. S. Severtsov. 2000. Survival and growth of brown frog juveniles in their dispersal away from breeding pond. Zoologichesky Zhurnal **79**:729-741.

MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Monographs in population biology. Princeton University press, Princeton.

McGarigal, K., and S. A. Cushman. 2002. Comparative evaluation of experimental approaches to the study of habitat fragmentation effects. Ecological Applications **12**:335-345.

Mazerolle, M. J., and A. Desrochers. 2005. Landscape resistance to frog movements. Canadian Journal of Zoology-Revue Canadienne De Zoologie **83**:455-464.

Moran, E. F. 1993. Deforestation and land use in the Brazilian Amazon. Human Ecology **21**:1-21.

Morellato, L. P. C., and C. F. B. Haddad. 2000. Introduction: The Brazilian Atlantic Forest. Biotropica **32**:786-792.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature **403**:853-858.

Palen, W. J., D. E. Schindler, M. J. Adams, C. A. Pearl, R. B. Bury, and S. A. Diamonds. 2002.

Optical characteristics of natural waters protect amphibians from UV-B in the US Pacific Northwest. Ecology **83**:2951-2957.

Pough, F. H. 1980. Advantages of ectothermy for tetrapods. American Naturalist **115**:92-112.

Pounds, J. A., M. P. L. Fogden, and J. H. Campbell. 1999. Biological response to climate change on a tropical mountain. Nature **398**:611-615.

Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, and B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature **439**:161-167.

Preest, M. R., and F. H. Pough. 1989. Interaction of temperature and hydration on locomotion of toads. Functional Ecology **3**:693-699.

Preest, M. R., and F. H. Pough. 2003. Effects of body temperature and hydration state on organismal performance of toads, *Bufo americanus*. Physiological and Biochemical Zoology **76**:229-239.

Radambrasil – Ministério de Minas e Energia. 1983. Projeto Radambrasil: Levantamento de Recursos Naturais. Vol. 32. IBGE.

Räsänen, K., A. Laurila, and J. Merila. 2003. Geographic variation in acid stress tolerance of the moor frog, *Rana arvalis*. I. Local adaptation. Evolution **57**:352-362.

Relyea, R. A. 2005. The lethal impact of roundup on aquatic and terrestrial amphibians. Ecological Applications **15**:1118-1124.

Rittenhouse, T. A. G., and R. D. Semlitsch. 2006. Grasslands as movement barriers for a forest-associated salamander: Migration behavior of adult and juvenile salamanders at a distinct habitat edge. Biological Conservation **131**:14-22.

Rogowitz, G. L., M. Cortes-Rivera, and K. Nieves-Puigdoller. 1999. Water loss, cutaneous resistance, and effects of dehydration on locomotion of *Eleutherodactylus* frogs. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology **169**:179-186.

Rothermel, B. B. 2004. Migratory success of juveniles: A potential constraint on connectivity for pond-breeding amphibians. Ecological Applications **14**:1535-1546.

Rothermel, B. B., and R. D. Semlitsch 2002. An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. Conservation Biology **16**:1324-1332.

Semlitsch, R. D., D. E. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in Ambystoma talpoideum. Ecology **69**:184-192

Silva, W. G., J. P. Metzger, S. J. Simões, C. Simonetti. 2006. Relief influence on the spatial distribution of the Atlantic Forest cover at the Ibiúna Plateau, SP. Brazilian Journal of Biology, in press.

Silvano, R. A. M., S. Udvardy, M. Ceroni, and J. Farley. 2005. An ecological integrity assessment of a Brazilian Atlantic Forest watershed based on surveys of stream health and local farmers' perceptions: implications for management. Ecological Economics **53**:369-385.

Sokal, R. R., and F. J. Rohlf. 2000. Biometry: the Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, New York.

Spight, T. M. 1968. Water economy of salamanders - evaporative water loss. Physiological Zoology **41**:195-&.

SYSTAT. 2002. SYSTAT for Windows, version 10.2.01. SYSTAT. Richmond, California.

Tabarelli, M., L. P. Pinto, J. M. C. Silva, M. Hirota, and L. Bede. 2005. Challenges and opportunities for Biodiversity conservation in the Brazilian Atlantic Forest. Conservation Biology **19**:695-700.

Tocher, M. D., C. Gascon, and J. Meyer. 2001. Community composition and breeding success of Amazonian frogs in continuous forest and matrix habitat aquatic sites. Pages 235-247 in R. O. Bierregaard Jr, C. Gascon, T. E. Lovejoy, and R. C. G. Mesquita, editors. Lessons from Amazonia: The Ecology and Conservation of a Fragmented Forest. Yale University Press, New Haven.

Urbina-Cardona, J. N., M. Olivares-Perez, and V. H. Reynoso. 2006. Herpetofauna diversity and microenvironment correlates across a pasture-edge-interior ecotone in tropical Rainforest fragments in the Los Tuxtlas Biosphere Reserve of Veracruz, Mexico. Biological Conservation **132**:61-75.

Viana, V. M., A. A. J. Tabanez, and J. L. Batista. 1997. Restoration and management of fragmented landscapes. Pages 347-365 in W. F. Laurance, and R. O. Bierregaard, editors. Tropical forest remnants: ecology, management and conservation of fragmented communities. University of Chicago Press, Chicago.

Vredenburg, V. T. 2004. Reversing introduced species effects: Experimental removal of introduced fish leads to rapid recovery of a declining frog. Proceedings of the National Academy of Sciences of the United States of America **101**:7646-7650.

Wilcox, B. A., and D. D. Murphy. 1985. Conservation strategy: the effects of fragmentation on extinction. The American Naturalist **125**:879-888.

Webb, T. J., F. I. Woodward, L. Hannah, and K. J. Gaston. 2005. Forest cover-rainfall relationships in a biodiversity hotspot: The Atlantic Forest of Brazil. Ecological

Applications **15**:1968-1983.

Welsh, H. H., and L. M. Ollivier. 1998. Stream amphibians as indicators of ecosystem stress: A case study from California's redwoods. Ecological Applications **8**:1118-1132.

Werner, E. E., and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size structured populations. Annual Review of Ecology and Systematics **15**:393-425.

Young, B. E., K. R. Lips, J. K. Reaser, R. Ibanez, A. W. Salas, J. R. Cedeno, L. A. Coloma, S. Ron, E. La Marca, J. R. Meyer, A. Munoz, F. Bolanos, G. Chaves, and D. Romo. 2001. Population declines and priorities for amphibian conservation in Latin America. Conservation Biology **15**:1213-1223.

Zar, J. H. 1999. Biostatistical Analysis, 4th edn. Prentice Hall, Englewood Cliffs.

Capítulo II

Litter-amphibian guilds in a severely fragmented landscape of Brazilian Atlantic Forest

Abstract

Amphibians with different reproductive modes and with different habitat preferences can use the landscape in different ways. In this study, we describe the community structure of litter-amphibians of a severely fragmented landscape of Atlantic Rainforest, grouping the species in three guilds: (i) forest-associated species with direct development or endotrophic terrestrial larvae, (ii) forest-associated species with aquatic larvae, and (iii) grass-field-associated species with aquatic larvae. Our study area is a severely fragmented landscape of Atlantic Forest in southeastern Brazil. In this landscape we evaluate the frequencies of each guild with pit-fall traps in five habitat categories of forest fragments with streams, and forest fragments without streams, including potential breeding-migration routes at matrix. Forest species with aquatic larval stage were more abundant in forest fragments with streams than forest fragments without streams, whereas species with direct development or endotrophic terrestrial larvae species where equally registered at forests with and without streams, and avoided the matrix. Forest species with aquatic larval stage were clearly associated with matrix habitats, suggesting breeding migrations through the matrix at least in absence of reproductive sites at forest fragments. Our results suggest that the matrix modified habitats surrounding forest fragments without breeding sites are likely to have pervasive effects on forest amphibians with aquatic larvae. Forest-associated species with aquatic larvae, that are more prone to cross hostile and unfamiliar habitats, should pay high demographic prices for these dangerous migrations. The species that avoid the matrix certainly experience long term genetic harms due to lower demographic contributions of immigrants, and also via stochastic population collapses. Nevertheless, the impacts of induced migrations through matrix habitat may result in immediate declines on populations of “matrix tolerant species” as many forest-associated amphibians with aquatic larval stage.

Introduction

The expansion of land use by human populations results in the fragmentation of natural landscapes (Wilcox & Murphy 1985). This is recognized as one of the most important threats to biodiversity, especially in tropical regions where diversity is high and forests are being lost at alarming rates. Since fragmented landscapes are composed by mosaics of different habitats with varying quality for fauna, understanding how species are affected by fragmentation requires information on their responses to the distinct habitats of the landscape, including the intervening matrix (Laurance & Yensen 1991, Malcolm 1991, Tocher *et al.* 1997, Gascon *et al.* 1999).

The matrix is an extensive area that surrounds patches of original habitat which can act as a barrier to gene flow and dispersion of the organisms that are confined to forest remnants (Wilcove *et al.* 1986). For this reason, the vulnerability of species in fragments has been assumed to be directly related to their ability to use the matrix habitats: those that tolerate severely degraded habitats are predicted to be less vulnerable, and vice-versa (Malcolm 1991, Laurance & Yensen 1991, Gascon *et al.* 1999). The explanations for the matrix vulnerability relationships is that populations of matrix tolerating species in fragments are bolstered by the genetic and demographic contributions of immigrants (Bown & Kodric-Bown 1977), originating either from other forest areas or the matrix itself. If such species disappear from the fragments they are also more likely to be reestablished by colonists.

Amphibian species with different reproductive modes use the matrix in different ways. While forest-associated species with aquatic larvae may be induced to carry out breeding migrations through the matrix to reproduce in water bodies, species with direct development or endotrophic terrestrial larvae could easily find adequate reproductive sites at leaf-litter in the forest. The effect of habitat loss and fragmentation on different guilds depends on their particular autoecology, movement patterns, habitat use, and ability to cope with biotic and abiotic microhabitat changes caused by disturbances (Pearman 1997, Green *et al.* 2001). Forest amphibians can use the matrix for different purposes such as feeding, traveling, dispersing, and reproduction (Gascon 1993, Tocher 1998, Gascon *et al.* 1999, Tocher *et al.* 2001). In this way, identifying guilds that better cope with the matrix habitats could help to understand how the forest loss and fragmentation affects the diversity of amphibians.

In this study, we described the community structure of litter-amphibians of a fragmented landscape of the Brazilian Atlantic Forest, by grouping the species in three guilds: (i) forest-associated species with direct development or endotrophic terrestrial larvae, (ii) forest-associated species with aquatic larvae, and (iii) matrix-associated species with aquatic larvae. Our working hypothesis is that these guilds differ in their responses to fragmentation, in which the need of differential breeding sites is a key factor. We test this hypothesis by comparing the abundances for each guild in forest fragments with streams and forest fragments without streams, including potential breeding-migration routes in the pasture matrix.

Methods

Study site

The study area is in the Brazilian Atlantic Forest domain, one of the most threatened biodiversity hot-spots (Myers *et al.* 2000). The high biodiversity of this biome, including about 300 endemic amphibian species (Haddad 1997), has been dramatically endangered by land-use, mostly by agriculture, cattle-raising, and urbanization (Viana *et al.* 1997, Dale & Pearson 1997, Dean 1997, Morellato & Haddad 2000, Tabarelli *et al.* 2005).

Our study area is a forest fragmented landscape in southeastern Brazil, located between the inland Mantiqueira mountain range and the coastal Serra do Mar mountain range (23°13' S, 45°20' W). To describe the area and define the studied fragments we used a 2002 land cover classification (5-meter resolution SPOT image), and hydrographic maps (IBGE 1:50.000). The current situation of streams was checked through an exhaustive field search.

The area is representative of the severe fragmentation suffered by the Atlantic Forest and since the 1930's is dominated by pasture matrix (59.8% of the cover), which occupies the most accessible, irrigated, and most productive areas of the landscape. Forests currently cover only 11.8% of the landscape, scattered in small and disturbed fragments. The other areas of the landscape are covered by early-growth forests (21.4%), *Eucalyptus* monoculture (3.7%), and urban areas (3.3%). The studied landscape covers 24,376 ha, with altitudes ranging from 756 to 1,080 m, and with 69 forest fragments ranging in size from 10 to 252.5 ha, located mainly in higher quotas of the terrain (forest fragments altitudinal mean = 843.54 m ± 7.95 SE, N = 100; pastures altitudinal mean = 815.81 m ± 7.74 SE, N = 100). Among the forest patches, only three are crossed by a permanent stream. The study area has 11.76% of forest, being that 9.79% of the drainage ditches are in natural forest cover. Excluding the first order drainages of the analysis, which are usually dry even in rainy seasons (personal field observation), just 8.56% of streams flow in the forests. In the studied landscape streams and microhabitats formed by them (e.g., pools, swamps) are indeed the main breeding resource for the species with aquatic larvae. In the present study the definition of a species as a stream breeder refers to species reproducing in rivulets and assoriated microhabitats.

The mean annual rainfall is 1,277 mm (ANA 2006) with the rainy season beginning in November with a mean rainfall of 112.4 mm. The rainy season reaches the monthly precipitation peak in January (230.4 mm), and finishes in March (155.8 mm). In the rest of the year the rainfall does not exceed 100 mm monthly, with the driest month being August (27.1 mm) (ANA 2006). The mean annual temperature is 20 °C, with the lowest mean in July (12 °C) and highest mean in February (27 °C) (Radambrasil 1983).

Frog guilds

We captured thirteen species totaling 504 individuals during the 2005/2006 rainy season. We used the available data on natural history of the sampled species to separate them in three distinct anuran guilds according to their habitat preferences and reproductive modes (Heyer *et al.* 1990, Haddad & Sazima 1992, Haddad 1997, Bertolucci 2001, Oliveira 2004, Dixo 2005). The first group includes all species with direct development or terrestrial endotrophic larvae and associated to forest habitats, such as the three species of Brachycephalidae family *Eleutherodactylus guentheri, E. binotatus, E. pavus* and *Leptodactylus marmoratus*. The second group includes forest-associated species with aquatic larval stage such as *Chaunus ornatus, C. ictericus, Proceratophrys boiei*, and *Leptodactylus mystacinus*. The last guild includes the matrix-associated species with aquatic larval stage, all very common species with high geographic ranges, such as *Leptodactylus furnarius, L. labyrinthicus, Physalaemus cuvieri, Elachistocleis ovalis,* and the introduced species *Lythobathes catesbeianus*.

Sampling design

We structured the sampling design comparing three forest fragments (> 10 ha) without streams (12.53, 17.42 and 42.34 ha) with the only three available forest fragments (> 10 ha) with streams in the studied landscape (19.27, 20.30, and 126.93 ha) (See chapter 1, Fig. 1A). At the fragments without streams we sampled three habitats: upland forest in the fragment (forest - F); the closest stream in the adjacent pasture matrix (matrix stream - MS), and the adjacent pasture matrix between the upland fragment and the matrix stream (matrix - M) (See chapter 1, Fig. 1B). In the fragments with streams we sampled in the upland forest (forest - F), and in the stream inside the forest (forest stream - FS) (See chapter 1, Fig 1C). The forest streams were all small second or first order creeks in steep drainages, 0.45 – 2.00 m wide and 10 – 50 cm deep. In the pasture matrix the sampled streams were all of second order, larger, (0.45 – 2.50 m wide and 15 – 50 cm deep) and in sanded flat valleys.

We sampled amphibians using pit-fall traps with drift fences adapted from Halliday (1996). Each trap consisted of a 45 m long and 0.6 m tall plastic fence supported by wooden stakes, intercepting four equidistant 60 l buried plastic buckets. All the traps of matrix were surrounded by wire fences 1.70 m high to avoid damages by cattle or horses.

To determine the position of the traps in forest patches we designed places randomly, but according to the following criteria: (i) the traps should be at least 30 m away from the fragment edge, (ii) they should be 70 m apart from each other, and (iii) all traps should be oriented by contour lines. The upland forest traps of the fragments with streams were installed in higher portions of the relief, at least 100 m from the forest stream.

For the installation of traps near the streams we randomized two points along the streambed and followed the criteria of: (i) each trap should be 70 m apart from the other stream trap; (ii) stream traps should be at maximum distance of 4 m from the streambed and with drift fence oriented parallel to the streambed; (ii) the forest stream traps (FS) should be at least 30 m from the fragment edge; the matrix stream traps (MS) were installed at the fragment side of the stream.

The matrix traps (between fragments and matrix streams - M) were oriented parallel to the fragment edge and matrix stream.

The sampling design comprehends three pairs of traps for each habitat category both in the fragments with streams (F, FS) and without streams (F, M, MS), totaling 30 traps. The traps remained open for 10 days.month during five months (November 2004 to March 2005), totaling an effort of 1500 traps.day.

For each trapped individual we identified the species and sex. Also, each captured individual was weighed with portable scales to the nearest 0.1 g, marked, and released five meters away from the trap. The individual marking was made by single toe-clipping, which registered the census and habitat (forest or matrix) (adapted by Halliday 1996). No recaptures among censuses or traps were recorded and for the very few individuals recaptured at the same trap and on the same census we used only their initial capture data in the analysis.

Data analysis

We performed one-way ANOVAs to compare: (i) number of captures, (ii) species richness, and (iii) species diversity (Simpson) between the five habitat categories of forest fragments with streams (F, FS) and forest fragments without streams (F, M, MS). These analyses were run in the package SYSTAT 10.2 (SYSTAT 2002).

We performed a Detrended Correspondence Analysis (DCA) to detect amphibian responses to the five environmental classes sampled. Using Multi Variate Statistical Package (MVSP) 3.12 software (Kovach, 2001), we examined the relationships between species distribution in the two habitat categories of fragments with streams (F, FS), and the three habitat categories of forest fragments without streams (F, M, MS). We used square root transformations for abundance data.

Results

Community attributes and habitat selection

In the forest fragments with streams, seven species were recorded in forest traps (F), and six species were recorded in forest stream traps (FS). In forest fragments without streams, seven species were recorded in forest traps (F), and the neighboring matrix habitats presented the highest accumulated species richness with 10 species recorded in the matrix (M), and 9 species in the matrix streams (MS). The three species recorded exclusively in the forest fragments were all species with direct development or terrestrial endotrophic larvae (*Eleutherodactylus binotatus, E. guentheri,* and *Leptodactylus marmoratus*), while three species with aquatic larval stage (*Leptodactylus furnarius, Elachistocleis ovalis,* and *Lythobates catesbeianus*) were exclusively recorded in the matrix. Three forest-associated species with aquatic larval stage (*Chaunus ictericus, C. ornatus,* and *Leptodactylus mystacinus*) were found in all five habitat categories. The forest-associated species contributed to the general increase in species richness in the matrix habitats. *Chaunus ornatus* was the most common species, accounting for 34.92% of all captures (Table 1).

The mean number of captures in the three categories of forest habitats ranged from 26.67 ± 11.566 SE to 48.67 ± 9.684 SE, and from 26.67 ± 6.667 SE to 29.333 ± 2.404 SE in the two categories of matrix habitats, with no significant differences in abundance between each one of the five habitat categories ($F_{4,10} = 1.576$, $r^2 = 0.378$, $p = 0.255$). We recorded a higher number of species in the matrix (M) and matrix streams (MS). However, species richness and species diversity showed no statistical differences between habitats (species richness $F_{4,10} = 1.671$, $r^2 = 0.410$, $p = 0.232$; species diversity $F_{4,10} = 2.682$, $r^2 = 0.518$, $p = 0.094$).

Table 1 Matrix representing observation frequencies of frog species and their natural history traits as recorded at five habitat categories of a fragmented landscape in Brazilian Atlantic Forest. The values are mean ± (SE).

Guild	Species	Family	Size	Forest fragments with stream		Forest fragments without stream			Total of records
				Forest (F)	Forest stream (FS)	Forest (F)	Adjacent Matrix (M)	Adjacent Matrix Stream (MS)	
FDD	*Eleutherodactylus binotatus*	Bra	M			13.00 ± 8.145			39
FDD	*Eleutherodactylus guentheri*	Bra	M	1.33 ± 0.667	16.67 ± 8.212	0.67 ± 0.333	0.33 ± 0.333		57
FDD	*Eleutherodactylus parvus*	Bra	S			0.67 ± 0.667			2
FDD	*Leptodactylus marmoratus*	Lep	S	0.33 ± 0.333	0.33 ± 0.333				2
FAL	*Chaunus ictericus*	Buf	L	0.33 ± 0.333	2.00 ± 1.528	0.67 ± 0.333	3.33 ± 0.333	5.00 ± 2.082	34
FAL	*Chaunus ornatus*	Buf	L	20.00 ± 1.155	17.33 ± 7.055	11.00 ± 2.646	4.00 ± 0.557	6.33 ± 1.453	176
FAL	*Proceratophrys boiei*	Cyc	L	8.00 ± 2.646	8.67 ± 5.364		0.67 ± 0.667	2.33 ± 1.856	59
FAL	*Leptodactylus mystacinus*	Lep	L	6.00 ± 2.000	3.67 ± 2.028	0.33 ± 0.333	3.00 ± 0.577	5.33 ± 1.764	55
MAL	*Leptodactylus furnarius*	Lep	M				0.33 ± 0.333	1.33 ± 0.882	5
MAL	*Leptodactylus labyrinthicus*	Lep	L			0.33 ± 0.333	0.33 ± 0.333	0.67 ± 0.667	4
MAL	*Physalaemus cuvieri*	Lei	S	0.67 ± 0.333			9.33 ± 6.984	4.33 ± 1.333	43
MAL	*Elachistocleis ovalis*	Mic	M				0.33 ± 0.333	0.67 ± 0.333	3
MAL	*Lithobates catesbeianus* *	Ran	L				5.00 ± 2.000	3.33 ± 2.404	25
	Number of captures			36.67 ± 0.667	48.67 ± 9.684	26.67 ± 11.566	26.67 ± 6.667	29.33 ± 2.404	***504***
	Species richness			5.00 ± 1.000	4.67 ± 0.667	4.00 ± 1.528	6.33 ± 0.882	7.00 ± 0.000	***13***

The habitat categories are listed in Table 1. Guild: FDD, forest-associated species with direct development or with terrestrial endotrophic larvae; FAL, forest-associated aquatic larvae species; MAL, matrix-associated aquatic larvae species (Haddad & Prado 2005). Family: Brachycephalidae, (Bra); Bufonidae, (Buf); Cycloramphidae, (Cyc); Leptodactylidae, (Lep); Leiuperidae, (Lei); Microhylidae, (Mic); Ranidae, (Ran); Classification follows (Frost *et al*. 2006). Size (snout-vent length): S, small species (< 31 mm); M, medium species (31-50 mm); L, large species (> 50 mm). * Exotic species.

We recorded a great variation in number of captures of direct development species between sample sites. Even with no statistical differences between all the five habitat categories ($F_{4,10} = 2.388$, $r^2 = 0.489$, $p = 0.120$), only one individual of the guild was recorded toward the matrix (Fig. 1A). Forest species with aquatic larval stage were more abundant in forest fragments with streams than in forest fragments without streams ($F_{4,10} = 24.500$, $r^2 = 0.907$, $p < 0.001$). Nevertheless, they were highly recorded in the matrix and matrix streams (Fig. 1B). The matrix species were more abundant in the matrix habitats ($F_{4,10} = 3.925$, $r^2 = 0.611$, $p = 0.036$) (Fig. 1C).

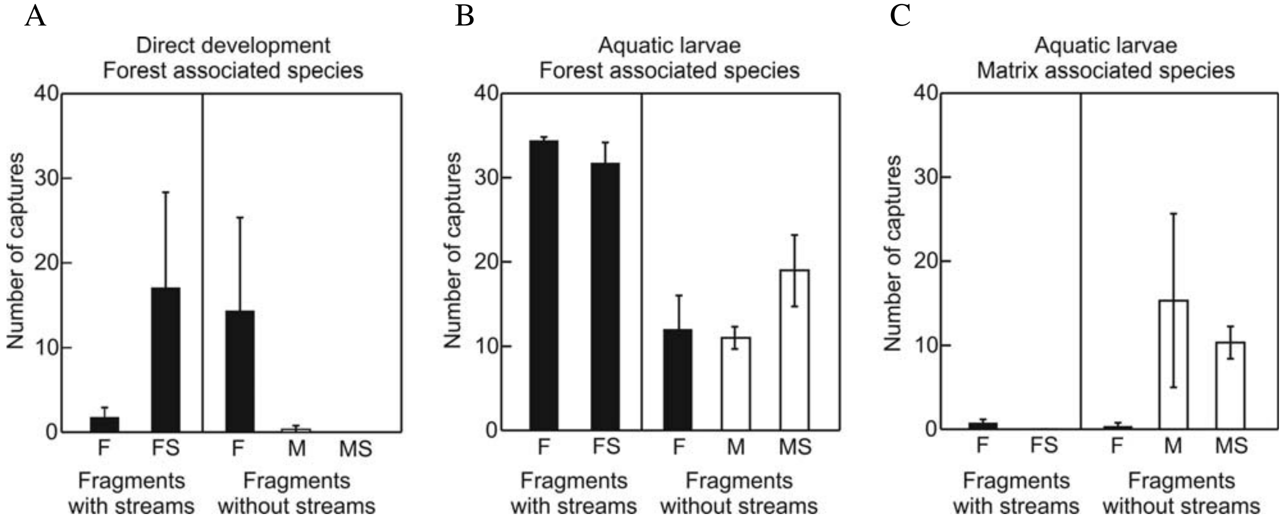


Figure 1 Mean number of captures in forest fragments with streams: Forest, F; Forest stream, FS. Number of captures in forest fragments without streams: Forest, F; Adjacent grass field matrix, M; Adjacent matrix stream, MS. Black bars represents forest formations; empty bars represents matrix habitats. The vertical lines represent ± 1 (SE).

Frog guilds

The three guilds were clearly distinct in a Detrended Correspondence Analysis (DCA). The Axes 1 and 2 together accounted for 37.14 % of the total inertia (31.57 % for Axis 1, 5.57 % for Axis 2). The first axis of the DCA clearly showed a gradient of habitat use between forest-exclusive species and matrix-exclusive species (Fig. 2). All direct development species or species with endotrophic terrestrial larvae were consistently associated to forest formations, with low tolerance to matrix habitats. However, forest species with aquatic larval stage were also clearly associated with matrix habitats, which represent reproductive migrations through the matrix at least in absence of reproductive sites in forest fragments. All matrix species were strongly associated to the grass field matrix.

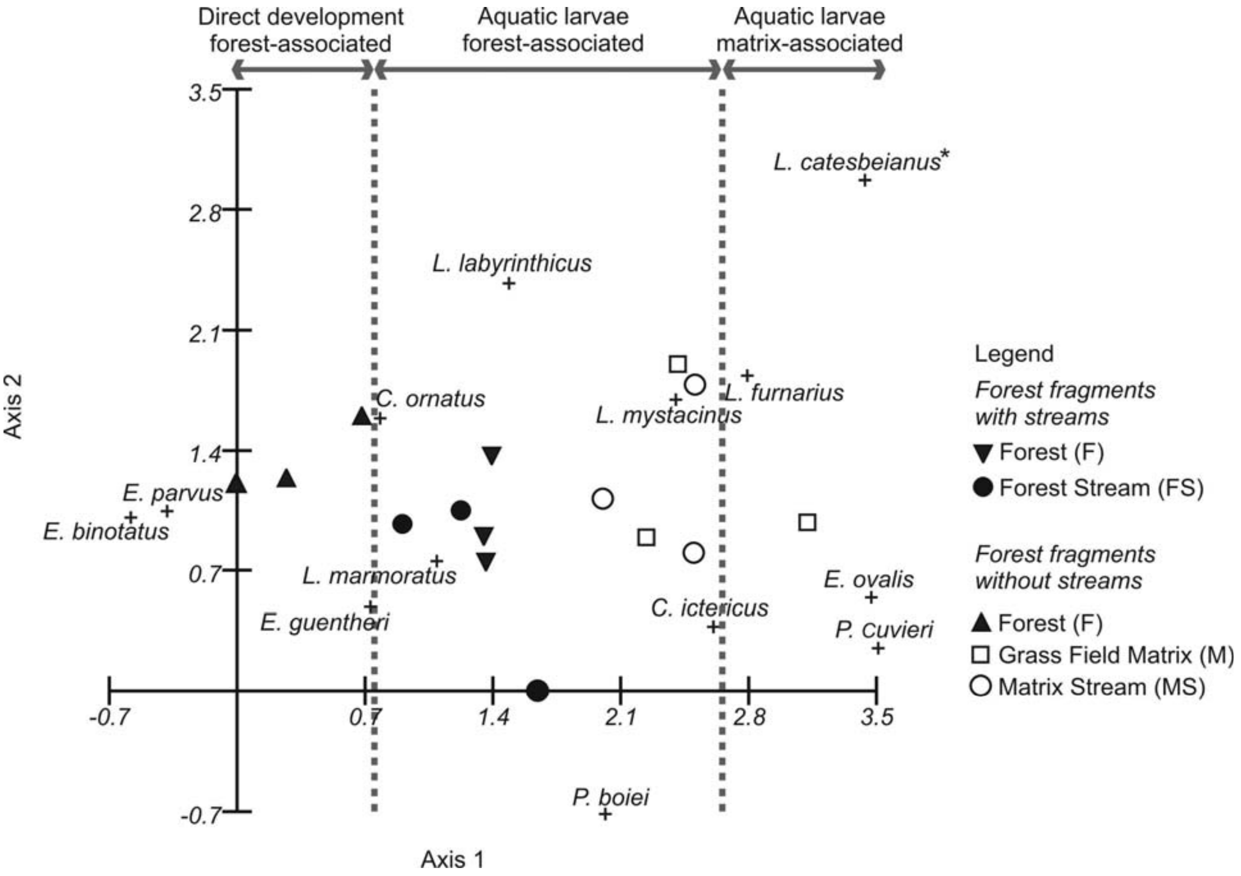


Figure 2 Detrended correspondence analysis (DCA) of the three amphibian guilds. The orientation and length represents the association, direction and strength between the habitats and each species (ter Braak 1987). Amphibian species are represented by +. Geometric figures represent the five habitat categories: black fill represents forest formations; empty fill represents matrix habitats. Dotted lines delimit an approximated arbitrary boundary between each frog guild.

Discussion

Species richness and population sizes of forest-dependent animals usually decline as a result of forest loss and fragmentation (Franklin & Forman 1987). Severely fragmented landscapes are expected to have higher species richness and species diversity on the remnants of natural habitat, while the anthropogenic matrix acts as a selective filter where only a little fraction of primary-forest species are able to persist or even cross (Laurance & Yensen 1991, Malcolm 1991, Gascon *et al.* 1999). Aggressive species can also spread via matrix and occupy forest patches, impacting the original community.

Surprisingly, our results show that the matrix could be the richest habitat in case of severe fragmentation by joining two guilds in some particular fragmented landscapes where there is high mismatch between forest fragments and aquatic habitats. Our findings highlighted that the matrix habitats surrounding fragments without breeding sites are likely to have wide-ranging effects on forest amphibians with aquatic larvae (See Chapter I). In contrast with most of species reproducing on the forest floor, aquatic-breeding forest amphibians have seasonal breeding migrations between terrestrial and aquatic habitats (Sinsch 1990, Rittenhouse & Semlitsch 2006). When they cannot detect adequate breeding sites in forest remnants, they may be induced to cross the matrix habitats for breeding. This could explain why forest-associated amphibians with aquatic larvae are recorded more often into the matrix habitats than forest-associated species with direct development (Pearman 1997, Oliveira 2004, Dixo 2005, Urbina-cardona *et al.* 2006), and why the matrix was the habitat with the higher number of species recorded in our study.

The different responses of frog guilds

We found that the three amphibian guilds differed in species abundance among habitats. Even the grass-field matrix has been considered by several studies as a high quality habitat for anuran reproduction because of the presence of artificial ponds and lakes (Baker & Halliday 1999, Knutson *et al.* 2004). Our results demonstrated that the matrix was not necessarily an ideal habitat for the maintenance of forest-associated species with aquatic larvae and species reproducing on the forest floor. It seems paradoxical that the matrix can have higher amphibian richness than forest, but this is explained by the differences of amphibian's life history between guilds and a particular landscape configuration of mismatched aquatic and terrestrial habitats.

Similar to other organisms, studies of forest fragmentation involving amphibians have been concentrated on the patches of the natural habitat, and the habitat use of the matrix has long been ignored (Tocher *et al.* 1997, Tocher 1998, Gascon *et al.* 1999, Urbina-cardona *et al.* 2006). In these studies, the guild of species with aquatic larvae better tolerated the matrix, but showed a large amount of records of population suppressions. Nonetheless, direct development species, which usually avoided open habitats, may show lower chances to get extinct. In a long-term multitaxonomic study involving experimental cutting and isolation of forest fragments from continuous forest in the Amazon Forest (Biological Dynamic of Forest Fragments Project - BDFFP), frogs showed the largest proportion (80%) of nominally primary-forest species detected in pasture and/or regrowth forest (Gascon *et al.* 1999). Numerous studies recorded primary-forest amphibians breeding in the matrix (Tocher *et al.* 1997, Tocher 1998), mainly species with aquatic larvae previously characterized as primary-forest species (Zimmerman & Rodrigues 1990, Gascon 1991) capable of using other types of habitat for reproduction (Gascon 1993). For example, the forest species *Epipedobates femoralis* used pools outside the forest, in second growth, at the same frequency as pools inside the primary forest (Gascon 1993). In the same experimental study (BDFFP), eight species were vanished from the fragments after isolation from continuous forest. From these, seven species had aquatic larvae and at least five had reproduction associated to streams (Tocher *et al.* 1997). In a tropical Rainforest of Mexico, Urbina-cardona *et al.* (2006) showed that species that avoided matrix habitats have mainly direct development, while forest-associated species with aquatic larvae were regularly recorded in the matrix habitat, probably searching for breeding sites.

In our study, stream-breeding forest species also had three times higher number of captures in fragments with streams than in fragments without streams, suggesting high mortality where there was compulsory migration for reproduction. They certainly are a subset of the original community that endures even with the adversities of reproduction at impacted breeding sites. For instance, in a pristine reserve only 15 km away from our study site 12 additional forest-associated species with aquatic larvae were recorded (L. O. M. Giasson & C. F. B. Haddad, unpublished data). This suggests that this is the more threatened guild because much of these species should pay high demographic costs for risky migrations. Nonetheless, direct development species and matrix-associated species may suffer much less from the breeding migrations because they can easily find adequate breeding sites in their preferred habitats. Therefore, we believe that the demographic costs of migrations through hostile habitats could have higher immediate impacts on population viability than could the genetic and demographic restrictions imposed by isolation on patchy populations. In summary, we propose that the compulsory breeding migrations through hostile habitats is one of the most important reasons why forest species with aquatic larval stage have a higher disproportional number of declining species compared to direct development species worldwide (Lips *et al.* 2003, Hero & Morrison 2004, Bustamante *et al.* 2005, Hero *et al*. 2005, Eterovick *et al.* 2005).

The repercussions of the matrix-tolerance model

The matrix-tolerance model, developed from studies of small mammal communities (Laurance & Yensen 1991, Malcolm 1991), states that the vulnerability of species in fragments is assumed to be directly related to their ability to use the matrix habitats: those that tolerate heavily degraded habitats are predicted to be less vulnerable, and vice-versa. The explanations for the matrix vulnerability relationships is that populations of matrix-tolerating species in fragments are bolstered by the genetic and demographic contributions of immigrants (Bown & Kodric-Bown 1977), originating either from other forest areas or from the matrix itself. If such species disappear from the fragments, they are also more likely to be reestablished by colonists.

Moreover, even the matrix-tolerance model having enormous conservation value as predictor of species vulnerability, it does not encompass crucial differences in landscape configuration such as the connection between forest patches and aquatic breeding sites. The model is extremely sensible to the geographical differences of sampling sites in the matrix: if the sampled site is on a breeding migration route to an aquatic breeding site, the model will indicate that all species that cross through the matrix will have lower chances to disappear from the patch. However, many of these species may suffer much more from migrations and reproductive failures in the matrix than species that experiences the adversities of population isolation, such as inbreeding and stochastic population collapses in forest remnants.

According to the matrix-tolerance model, direct development forest species should be the guild with the highest susceptibility to decline, whereas forest-associated species with aquatic larvae could maintain higher demographic viability and gene flow between patchy populations via dispersion through the matrix. Matrix-associated species generally have a high geographic range and are usually common species, being the guild with the lowest probability to decline. However, the costs of migration per guild do not follow the same gradient of vulnerability of the matrix-tolerance model. As direct development forest species usually do not cross through the matrix, the costs they spend with migrations tend to zero. Forest-associated species with aquatic larvae can be induced to migrate through, and/or reproduce in unfamiliar matrix, suffering very much from the migrations and potential reproductive failures.

Concluding remarks

In the studied landscape the guilds differing in habitat preferences and reproductive modes used the landscape in clearly different ways. As the guild that suffers more worldwide is the group of species that better "tolerated" the matrix habitats, we suggest that the costs of genetic and demographic restrictions imposed by isolation (assumptions of matrix-tolerance model) have to be balanced with the immediate costs of compulsory migrations through unfamiliar hostile habitats. Hence, the temporal scale of the impacts should also be considered, since the effects of deleterious breeding migrations followed by reproductive failures have much more immediate consequences when compared to the effects of stochastic crashes and genetic bottlenecks. Finally, the species that avoid the matrix certainly experience long-term genetic harms due to lower demographic contributions of immigrants (Bown & Kodric-Bown 1977). Nevertheless, the impacts of induced migrations through matrix habitats may result in immediate declines on populations of matrix-tolerant species such as many forest-associated amphibians with aquatic larval stage.

References

ANA, Agência Nacional de Águas. 2006. Pluviosity database. Agência Nacional de Águas, Brasília, Brazil.

Baker, J. M. R., and T. R. Halliday. 1999. Amphibian colonization of new ponds in an agricultural landscape. Herpetological Journal **9**:55-63.

Bertoluci, J. A. 2001. Anfíbios anuros. Pages 159-168 in C. Leonel, editor. Intervales/ Fundação para a Conservação e Produção Florestal do Estado de São Paulo. A Fundação, São Paulo.

Brown, J. H., and A. Kodric-Brown. 1977. Turnover rates in insular biogeography: Efect of immigration on extinction. Ecology **58**:445-449.

Bustamante, M. R., S. R Ron, and L. A. Coloma. 2005. Cambios em la diversidad em siete comunidades de anuros en los Andes de Ecuador. Biotropica **37**:180-189.

Dale, V. H., and S. M. Pearson. 1997. Quantifying habitat fragmentation due to land-use change in Amazonia. Pages 400–410 in W. F. Laurance, and R. O. Bierregaard, editors. Tropical Forest Remnants: Ecology, Management and Conservation of Fragmented Communities. University of Chicago Press, Chicago.

Dean, W. 1997. A ferro e fogo: a história e a devastação da Mata Atlântica brasileira. Companhia das Letras, São Paulo.

Dixo, M. B. O. 2005. Diversidade de sapos e lagartos de serrapilheira numa paisagem fragmentada do Planalto Atlântico de São Paulo. Universidade de São Paulo – USP, 180p.

Eterovick, P. C., A. Carnaval, D. M. Borges-Nojosa, D. L. Silvano, M. V. Segalla, and I. Sazima. 2005. Amphibian declines in Brazil: An overview. Biotropica **37**:166-179.

Franklin, J. F., and R. T. T. Forman. 1987. Creating landscape patterns by forest cutting: ecological consequences and principles. Landscape Ecology **1:**5-18.

Frost, D. R., T. Grant, J. Faivovich, R. H. Bain, A. Haas, C. F. B. Haddad, R. O. De Sa, A. Channing, M. Wilkinson, S. C. Donnellan, C. J. Raxworthy, J. A. Campbell, B. L. Blotto, P. Moler, R. C. Drewes, R. A. Nussbaum, J. D. Lynch, D. M. Green, and W. C. Wheeler. 2006. The amphibian tree of life. Bulletin of the American Museum of Natural History:8-+.

Gascon, C. 1991. Populaional-level and community-level analyses of species occurrences of central Amazonian rain-forest tadpoles. Ecology **72**:1731-1746.

Gascon, C. 1993. Breeding-habitat use by 5 amazonian frogs in the forest edge. Biodiversity and Conservation **2**:438-444.

Gascon, C., T. E. Lovejoy, R. O. Bierregaard, J. R. Malcolm, P. C. Stouffer, H. L. Vasconcelos, W. F. Laurance, B. Zimmerman, M. Tocher, and S. Borges. 1999. Matrix habitat and species richness in tropical forest remnants. Biological Conservation **91**:223-229.

Giasson, L. O. M., and C. F. B. Haddad. 2006. Amphibians of Núcleo Santa Virgínia. Unpublished data.

Green, D. M., R. L. Carroll, and V. H. Reynoso. 2001. Patrones de extinción en los anfibios: pasado y presente. Pages 169–200 in Hernandez, H.M., Garcia-Aldrete, A. N., F. Álvarez, and M. Ulloa, Editors. Enfoque Contemporaneo para el Estudio de la Biodiversidad. Instituto de Biología, UNAM, Fondo de Cultura Economica, México.

Haddad, C. F. B. 1997. Biodiversidade dos Anfíbios No Estado de São Paulo. Pages 15-26 in R. C. M. Castro, C. A. Joly, and C. E. M. Bicudo, editors. Biodiversidade do Estado de São Paulo, Brasil: Síntese do Conhecimento ao Final do Século XX. FAPESP, São Paulo.

Haddad, C. F. B., and I. Sazima. 1992. Anfíbios anuros da Serra do Japi. Pages 188-211 in: P. Morellato, editor. História Natural da Serra do Japi. Ecologia e Preservação de uma Área Florestal no Sudeste do Brasil, Editora da UNICAMP, Campinas.

Haddad, C. F. B., and C. P. A. Prado. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic forest of Brazil. Bioscience **55**:207-217.

Halliday, T. R. 1996. Amphibians. Pages 205-216 in W. J. Sutherland, editor. Ecological Census Techniques: a Handbook. Cambridge University Press, Cambridge.

Hero, J. M., S. E. Williams, and W. E. Magnusson. 2005. Ecological traits of declining amphibians in upland areas of eastern Australia. Journal of Zoology **267**:221-232.

Hero, J. M., and C. Morrison. 2004. Frog declines in Australia: global implications. The Herpetological Journal **14**:175-186.

Heyer, W. R., A. S. Rand, C. A. G. Cruz, O. L. Peixoto, and C. E. Nelson. 1990. Frogs of Boracéia. Arq. Zool. São Paulo **31**: 231-410.

Knutson, M. G., W. B. Richardson, D. M. Reineke, B. R. Gray, J. R. Parmelee, and S. E. Weick. 2004. Agricultural ponds support amphibian populations. Ecological Applications **14**:669-684.

Kovach, W. L. 2001. MVSP - A MultiVariate Statistical Package, ver. 3.12. Kovach Computing Services, Pentraeth, Wales, U.K.

Laurance, W. F., and E. Yensen. 1991. Predicting the impacts of the edge effects in fragmented habitats. Biological Conservation **55**:77-92.

Lips, K. R., J. D. Reeve, and L. R. Witters. 2003. Ecological traits predicting amphibian population declines in Central America. Conservation Biology **17**:1078-1088.

Malcolm, J. R., 1991. The small mammals of Amazonian forest fragments: Pattern and process. Ph.D. thesis, University of Florida, Gainesville.

Morellato, L. P. C., and C. F. B. Haddad. 2000. Introduction: The Brazilian Atlantic Forest. Biotropica **32**:786-792.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature **403**:853-858.

Oliveira, S. H. 2004. Diversidade de anuros de serrapilheira em fragmentos de Floresta Atlântica e plantios de *Eucalyptus saligna* no município de Pilar do Sul, SP. Dissertação, USP, Piracicaba, 60p.

Pearman, P. B. 1997. Correlates of amphibian diversity in an altered landscape of Amazonian Ecuador. Conservation Biology **11**:1211-1225.

Radambrasil – Ministério de Minas e Energia. 1983. Projeto Radambrasil: Levantamento de Recursos Naturais. Vol. 32. IBGE – Bazil.

Rittenhouse, T. A. G., and R. D. Semlitsch. 2006. Grasslands as movement barriers for a forest-associated salamander: Migration behavior of adult and juvenile salamanders at a distinct habitat edge. Biological Conservation **131**:14-22.

Sinsch, U. 1990. Migration and orientation in anuran amphibians. Ethology Ecology & Evolution **2**:65-79.

SYSTAT. 2002. SYSTAT for Windows, version 10.2.01. SYSTAT. Richmond, California.

Tabarelli, M., L. P. Pinto, J. M. C. Silva, M. Hirota, and L. Bede. 2005. Challenges and opportunities for biodiversity conservation in the Brazilian Atlantic Forest. Conservation Biology **19**:695-700.

ter Braak, C. J. F. 1987. Ordination. Pages 91-173 in R. H. G. Jongman, C. J. F. ter Braak and O.

F. R. van Tongeren editors. Data analysis in community and landscape ecology. Pudoc, Wageningen.

Tocher, M., 1998. A comunidade de anfíbios da Amazônia central: diferenças na composição de espécies entre a mata primária e pastagens. Pages 219-232 in C. Gascon, and P. Moutinho, Editors. Floresta Amazônica: Dinâmica, Regeneração e Manejo. Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil.

Tocher, M. D., C. Gascon, and J. Meyer. 2001. Community composition and breeding success of Amazonian frogs in continuous forest and matrix habitat aquatic sites. Pages 235-247 in: R. O. Bierregaard Jr, C. Gascon, T. E. Lovejoy, and R. C. G. Mesquita, editors. Lessons from Amazonia: The Ecology and Conservation of a Fragmented Forest. Yale University Press, New Haven.

Tocher, M., Gascon, C., Zimmerman, B., 1997. Fragmentation effects on a central Amazonian frog community: a ten-year study. Pages 124-137 in Laurance, W. F., and R. O. Bierregaard Jr., Editors. Tropical Forest Remnants: Ecology, Management, and Conservation of Fragmented Communities. University of Chicago Press, Chicago, Illinois.

Urbina-Cardona, J. N., M. Olivares-Perez, and V. H. Reynoso. 2006. Herpetofauna diversity and microenvironment correlates across a pasture-edge-interior ecotone in tropical Rainforest fragments in the Los Tuxtlas Biosphere Reserve of Veracruz, Mexico. Biological Conservation **132**:61-75.

Viana, V. M., A. A. J. Tabanez, and J. L. Batista. 1997. Restoration and management of fragmented landscapes. Pages 347-365 in W. F. Laurance, and R. O. Bierregaard, editors. Tropical Forest Remnants: Ecology, Management and Conservation of Fragmented

Communities. University of Chicago Press, Chicago.

Wilcox, B. A., and D. D. Murphy. 1985. Conservation strategy: the effects of fragmentation on extinction. The American Naturalist **125**:879-888.

Wilcove, D. S., McLellan, C. H., and Dobson, A. P. 1986. Habitat fragmentation in the temperate zone. Pages 237–256 in M. E. Soulé, editor. Conservation Biology: The Science of Scarcity and Diversity, first ed. Sinauer Associates, Sunderland.

Zimmerman, B. L. and M. T. Rodrigues. 1990. Frogs, snakes, and lizards of the INPA-WWF Reserves near Manaus, Brazil. Pages 426-454 in A. H. Gentry, editor. Four Neotropical Rain-forests. Univiversity of Yale Press.

Capítulo III

Habitat-split and the global decline of amphibians

Amphibian populations are declining worldwide due to a number of putative causes[1-3]. Here, we provide evidence that *habitat-split* - the mismatch of the habitat of tadpoles from the habitat of their corresponding adults due to non-random deforestation patterns - caused dramatic population suppressions in the Brazilian Atlantic Forest hotspot and is potentially a relevant mechanism for such global biodiversity crisis.

Hypotheses regarding the population decline of amphibians have been discussed as a web of complex causes[1-3]. Among the most advocated factors affecting amphibian populations are the influence of agrochemical contaminants[4-6], introduction of exotic species[7-8], uv-b radiation effects[9], climate shifts[10-11], and emerging diseases affecting even areas apparently protected from human influence[12-13]. In addition, on top of the list of presumed causes for amphibian population declines are habitat loss and fragmentation[14]. It is frequently assumed that habitat loss and fragmentation affect amphibian populations and communities in the same way they affect any other taxa, following the well known mechanisms discussed in the theory of island biogeography[15], metapopulation and edge effect models[16], such as population isolation, inbreeding, and decrease in habitat quality. Here, we bring support for a new mechanism associated to habitat alteration that is of relevance for explaining the population decline of amphibians.

Here we define habitat-split as the mismatch of the habitat of juveniles from the habitat of their corresponding adults at the landscape scale. Habitat-split forces individuals to cross from one habitat to another looking for reproductive opportunities, food, and enemy-free sites. During this journey, they are frequently obliged to cross hostile anthropogenic habitats facing multiple hazards. In human-altered landscapes, habitat-split can be expected to cause a decrease in population abundance, species richness, and changes in community structure. Since many amphibian species change considerably their niche along the ontogeny[17], with larvae being generally dependent on aquatic habitats and post-metamorphics being associated to terrestrial habitats, the constrains attributed to habitat-split are predicted to have widespread negative effects in this taxon.

In a local landscape of Atlantic Forest with fragments inserted in a grass-field matrix, habitat-split was an important mechanism explaining spatial variation in amphibian population abundance[18]. Forest species with aquatic larval stage were three times less captured in fragments without streams than in fragments with their breeding habitats ($F_{[1,4]} = 52.812$, $r^2 = 0.930$, $p = 0.002$). This result can be attributed to compulsory breeding migrations of adults from the forest fragments without streams to grass-field streams in the beginning of the rainy season and the opposite flux of post-metamorphics and surviving adults in the end of the reproductive period; both exposing the migrating amphibians to inhospitable matrix conditions (Fig. 1). In contrast, forest species that lay their eggs directly on the leaf litter, having tadpoles that can survive in the absence of free water, or having direct development, showed no difference in abundance between fragments with streams and without streams ($F_{[1,4]} = 2.263$, $r^2 = 0.361$, $p = 0.263$), since they do not need to leave the forest to breed successfully.

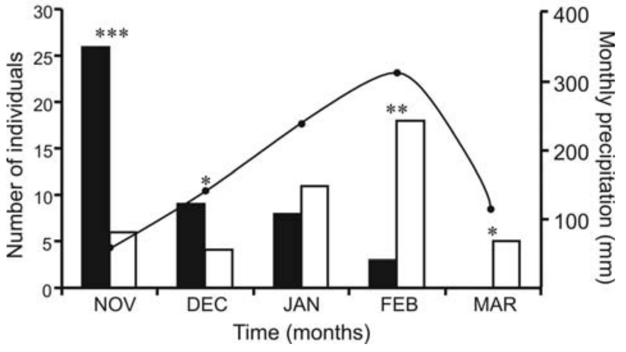


Figure 1 Migration pattern of forest associated species with aquatic larvae in a landscape of Brazilian Atlantic Forest with high level of habitat-split. Black bars represent forest fragment to grass-field matrix stream dislocation direction; white bars represent grass-field matrix stream to forest fragment dislocation direction. The line represents the monthly precipitation during the sampling period 2005/2006. Values of G: $* = p < 0.05$, $** = p < 0.005$, $*** = p < 0.001$.

Throughout several Atlantic Forest localities (Fig. 2), the habitat-split, measured by the percentage of the total length of all streams which do not flow through natural forest cover, is a good predictor of forest-associated litter-amphibian species richness ($F_{[1,11]} = 33.690$, $r^2 = 0.754$, $p < 0.001$). Species with different reproductive modes, however, responded differently to habitat-split; species dependent on water for breeding being more strongly affected that species with direct development or endotrophic terrestrial larvae (ANCOVA: $F_{[1,11]} = 18.339$, $p < 0.001$) (Fig. 3). A path-analysis allowing investigation of the multiple effect of habitat-split, habitat loss, and habitat fragmentation in a single model, indicated that habitat-split was the only factor which significantly affected the richness of species with aquatic larvae (Fig. 4A), whereas habitat loss had a marginal effect on the richness of species with direct development or endotrophic terrestrial larvae (Fig. 4B). A more complex model including the influences of the altitudinal range on species richness and habitat loss did not alter our findings.

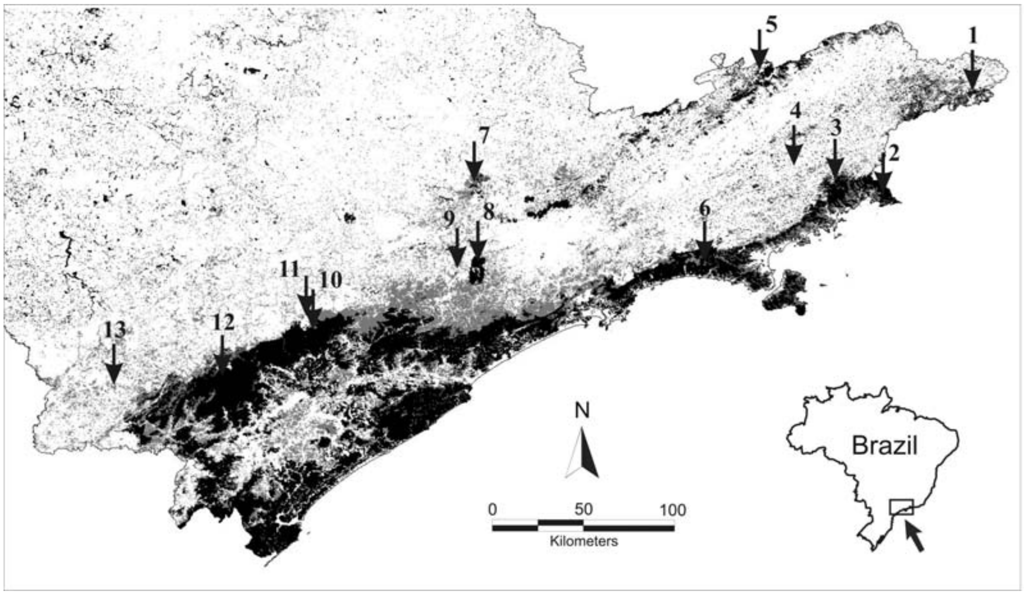


Figure 2 Map of natural forest cover of the State of São Paulo with black areas representing primary forests and grey areas representing second growth forests. Atlantic Forest sites with samples of leaf litter amphibians: (1) Bananal[27]; (2) Picinguaba[28]; (3) Núcleo Santa Virgínia[29]; (4) São Luis do Paraitinga[18]; (5) Campos do Jordão[30]; (6) Reserva de Boracéia[31]; (7) Serra do Japi[32]; (8) Reserva de Morro Grande[33]; (9) Cotia[33]; (10) Pilar do Sul – continuous forest[34]; (11) Pilar do Sul – fragmented forest[34]; (12) Parque Estadual de Intervales[35]; (13) Ribeirão Branco[36].

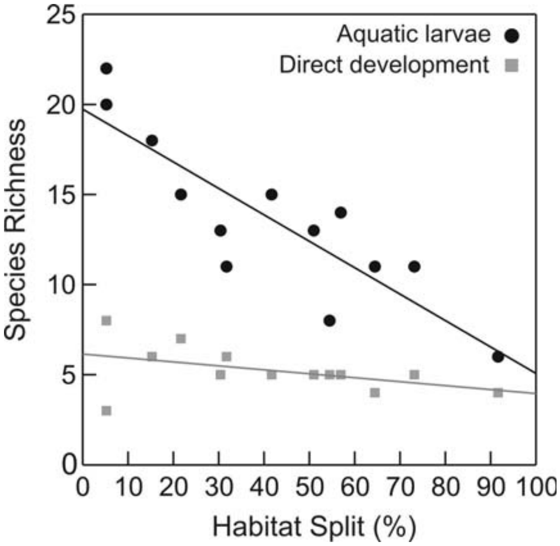


Figure 3 Linear regressions of forest-associated litter-amphibian species richness in 13 sites with different levels of habitat-split throughout the Brazilian Atlantic Forest.

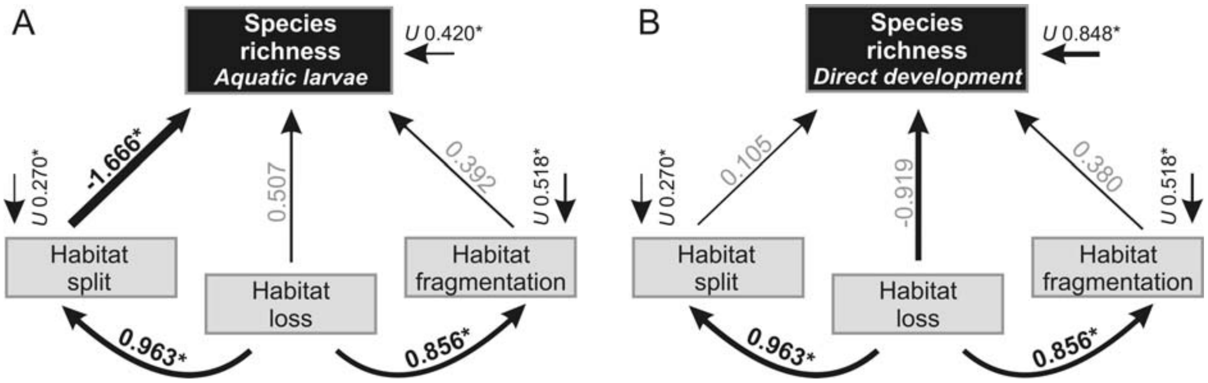


Figure 4 (A) Path analysis of the cross-correlated landscape attributes – habitat-split, habitat loss, and habitat fragmentation influencing species richness of aquatic larvae amphibians: model significant at 95% level; (B) Path analysis for species with direct development and endotrophic terrestrial larvae: model significant at 95% level. Path coefficients: * = $p < 0.001$.

Worldwide, species with aquatic larval stage are exactly the most endangered ones. For instance, in Eastern Australia, Tropical Andes, Brazilian Atlantic Forest, and Central America, forest species which require aquatic breeding sites have a disproportional number of declining species[19-23]. Perhaps not coincidently, those areas have topographic features that favor the mismatch between streams and forest remnants.

The intensity of habitat-split in a landscape depends fundamentally on how much of the original habitat was lost, on the degree of fragmentation of the remaining area, but above all, on the spatial distribution of the fragments regarding the water sources of the hydrographic basin. In order to reduce the negative impacts of habitat-split, specific laws concerning the protection of riparian zones and gallery forests should be created in countries where they are missing and urgently reinforced where proper legislation does exist. In the Brazilian Atlantic Forest, for instance, if the law insuring the protection of 30 m of riparian vegetation surrounding streams and rivers were adequately reinforced, the negative impact of habitat-split would be less pronounced and amphibian populations less threatened.

The maintenance and cleaning of the water is one of the main ecosystem services nature provides to man free of charge. In many countries, unplanned land use led human populations to destroy riparian forests leaving forest fragments far away from rivers and streams, causing a drastic reduction in the availability and quality of water. Nowadays, water is becoming a scarce resource for human populations worldwide. While the wealthiest live near enough water sources to use them for drinking, washing and recreation purposes, the poorer are increasingly being deprived of this elementary resource. Restoration programs aiming to re-build riparian and gallery forests are expected to improve the quality of life of most human populations and of a wide range of threatened amphibian species.

Methods

Spatial analysis and landscape metrics

We used only the available amphibian surveys of the Atlantic Forest from São Paulo State due to the minimal latitudinal interferences on species richness, and a wide range of landscape integrity classes. We used a land cover GIS database supplied by Programa Biota/Fapesp, Instituto Florestal/SMA[24] (LANDSAT 1998-99, 1:50.000), and hydrographic maps (IBGE 1:50.000)[25]. For each of the thirteen litter amphibian surveys across the Atlantic Forest[18,27-36] we extracted four landscape metrics: habitat loss, habitat fragmentation, habitat-split and the altitudinal range. The metrics were calculated for each circular buffer of a 15-km diameter defined from the center of each study site. Habitat loss was calculated by the percentage of non-natural vegetation cover on the circular buffer. Habitat fragmentation was calculated by the km forest edge per km^2 of natural forest on the circular buffer[26]. Habitat-split was calculated by the percentage of stream length flowing out of natural forest cover on the circular buffer. All drainages used on the analysis had a higher order than the first order streams. Altitudinal range was calculated by the higher minus the lowest altitudinal mark (meters) on the circular buffer.

Acknowledgements

We thank Luis Giasson and Itamar Martins for offering their unpublished records and João Giovanelli for suggestions and assistance during the analysis. C. G. Becker received a scholarship from the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP. C. R. Fonseca was supported by a fellowship from the Conselho Nacional de Desenvolvimento

Científico e Tecnológico (CNPq) and by UNISINOS. This project was supported by the Programa BIOTA/FAPESP and CNPq.

References

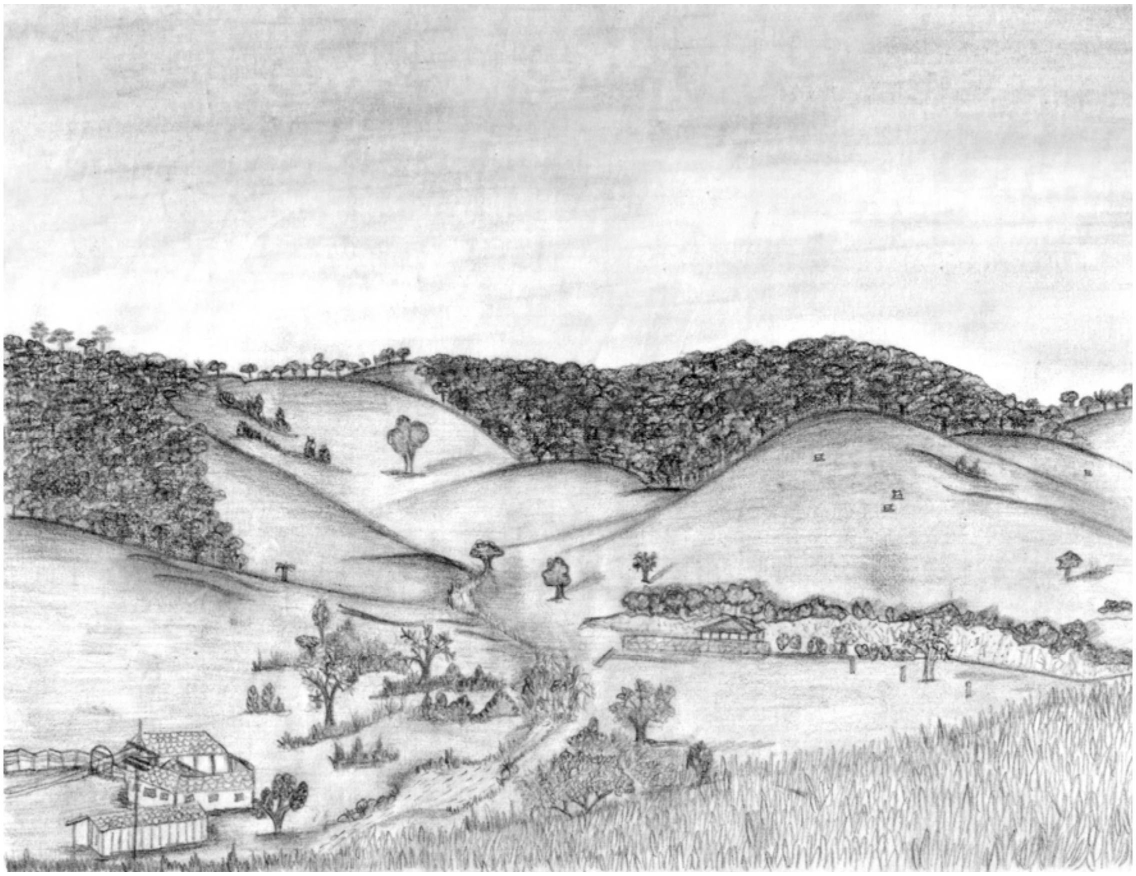
1. Alford, R. A. & Richards, S. J. Global amphibian declines: a problem in applied ecology. *Annu. Rev. Ecol. Syst.* **30**, 133-165 (1999).
2. Kiesecker, J. M., Blaustein, A. R., & Belden, L. K. Complex causes of amphibian population declines. *Nature* **410** (6829), 681-684 (2001).
3. Beebee, T. J. C. & Griffiths, R. A. The amphibian decline crisis: A watershed for conservation biology? *Biological Conservation* **125** (3), 271-285 (2005).
4. Boone, M. D., Semlitsch, R. D., Fairchild, J. F., *et al.* Effects of an insecticide on amphibians in large-scale experimental ponds. *Ecological Applications* **14** (3), 685-691 (2004).
5. Hayes, T. B., Collins, A., Lee, M., *et al.* Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proc. Natl. Acad. Sci. U. S. A.* **99** (8), 5476-5480 (2002).
6. Relyea, R. A. The lethal impact of roundup on aquatic and terrestrial amphibians. *Ecological Applications* **15** (4), 1118-1124 (2005).
7. Vredenburg, V. T. Reversing introduced species effects: Experimental removal of introduced fish leads to rapid recovery of a declining frog. *Proc. Natl. Acad. Sci. U. S. A.* **101** (20), 7646-7650 (2004).
8. Kiesecker, J. M. & Blaustein, A. R. Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth, and survival of native red-legged frogs (Rana aurora). *Conserv. Biol.* **12** (4), 776-787 (1998).
9. Blaustein, A. R. & Johnson, P. T. The complexity of deformed amphibians. *Frontiers in Ecology and the Environment* **1** (2), 87-94 (2003).
10. Pounds, J. A., Fogden, M. P. L., & Campbell, J. H. Biological response to climate change on a tropical mountain. *Nature* **398** (6728), 611-615 (1999).

11. Pounds, J. A., Bustamante, M. R., Coloma, L. A., *et al.* Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **439** (7073), 161-167 (2006).
12. Daszak, P., Cunningham, A. A., & Hyatt, A. D. Infectious disease and amphibian population declines. *Diversity and Distributions* **9** (2), 141-150 (2003).
13. Berger, L., Speare, R., Daszak, P., *et al.* Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc. Natl. Acad. Sci. U. S. A.* **95** (15), 9031-9036 (1998).
14. Cushman, S. A. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation* **128** (2), 231-240 (2006).
15. MacArthur, R. H. & Wilson, E. O. *The Theory of Island Biogeography*. (Princeton University press, Princeton, 1967).
16. Hanski, I. Metapopulation dynamics. *Nature* **396** (6706), 41-49 (1998).
17. Werner, E. E. & Gilliam, J. F. The Ontogenetic niche and species interactions in size structured populations. *Annu. Rev. Ecol. Syst.* **15**, 393-425 (1984).
18. Becker, C. G. Desconexão de hábitats e o declínio global dos anfíbios. Universidade Estadual de Campinas, Dissertation, (see chapter I). 2007.
19. Hero, J. M., Williams, S. E., & Magnusson, W. E. Ecological traits of declining amphibians in upland areas of eastern Australia. *Journal of Zoology* **267**, 221-232 (2005).
20. Bustamante, R. M, Ron, S. R., & Coloma, L. A. Cambios en la diversidad en siete comunidades de anuros en los Andes de Ecuador. *Biotropica* **37** (2), 180-189 (2005).
21. Eterovick, P. C., Carnaval, A., Borges-Nojosa, D. M., *et al.* Amphibian declines in Brazil: An overview. *Biotropica* **37** (2), 166-179 (2005).
22. Haddad, C. F. B. in *Lista da Fauna Brasileira Ameaçada de Extinção*, edited by A. B. Machado, C. S. Martins, & G. M. Drummond (Biodiversitas, Belo Horizonte, 2005), pp. 59-63.
23. Lips, K. R., Reeve, J. D., & Witters, L. R. Ecological traits predicting amphibian population declines in Central America. *Conserv. Biol.* **17** (4), 1078-1088 (2003).
24. Programa Biota/Fapesp & Instituto Florestal/SMA. Mapa de Vegetação Remanecente do Estado de São Paulo (2004).

25. Instituto Brasileiro de Geografia e Estatística – IBGE. Cartas topográficas 1:50.000 (1985).
26. Turner, M. G., Gardner, R. H. & O'Neil, R. V. *Landscape Ecology in Theory and Practice: Pattern and Process* (Springer, New York, 2001).
27. Zeher, H., Aguiar, E. & Pombal Jr., J. P. *Paratelmatobius gaigeae* (Cochran, 1938) re-discovered (Amphibia, Anura, Leptodactylidae), appendix I. *Arquivos do Museu Nacional* **63** (2), 321-328 (2005).
28. Hartmann, M. T. Biologia reprodutiva de uma comunidade de anuros (Amphibia) na Mata Atlântica (Picinguaba, Ubatuba, SP). Universidade Estadual Paulista Júlio de Mesquita Filho, 2004.
29. Giasson, L. O. M. & Haddad, C. F. B. Amphibians of Núcleo Santa Virgínia. Unpublished data.
30. Matins, I. A. Amphibians of Campos do Jorão. Unpublished data.
31. Heyer, W. R, Rand, A. S., Cruz, C. A. G., *et al.* Frogs of Boracéia. *Arc. Zool.* **31**, 231-410 (1990).
32. Haddad, C. F. B. & Sazima, I. in *História Natural da Serra do Japi. Ecologia e preservação de uma área florestal no sudeste do Brasil*, edited by P. Morellato (Editora da UNICAMP, Campinas, 1992), pp. 188-211.
33. Dixo, M. B. O. Diversidade de sapos e lagartos de serrapilheira numa paisagem fragmentada do Planalto Atlântico de São Paulo. Universidade de São Paulo, 2005.
34. Oliveira, S. H. Diversidade de anuros de serrapilheira em fragmentos de Floresta Atlântica e Plantios de *Eucalyptus saligna* no município de Pilar do Sul, SP. Universidade de São Paulo, 2004.
35. Bertoluci, J. A. in *Intervales/Fundação para a conservação do estado de São Paulo*, edited by C. Leonel (A Fundação, São Paulo, 2001), pp. 159-168.
36. Pombal Jr., J. P. & Haddad, C. F. B. Estratégias e modos reprodutivos de anuros (Amphibia) em uma poça permanente na serra de paranapiacaba, sudeste do Brasil, appendix I. *Papéis Avulsos de Zoologial* **45** (15), 201-213 (2005).

Considerações finais

No presente estudo, a desconexão entre os hábitats indispensáveis para o desenvolvimento dos anfíbios com fase larval aquática, tratada como *habitat-split,* foi sem dúvida, o principal atributo da paisagem explicando a abundância em nível populacional, a estrutura de comunidades, e a riqueza de anfíbios em larga escala geográfica. Anfíbios com desenvolvimento larval aquático fazem parte do grupo que apresenta as maiores taxas de declínio populacional no mundo inteiro e que mais sofre com a desconexão de habitat. Somente as espécies deste grupo são induzidas a migrações através de ambientes hostis e à reprodução em corpos d'água impactados quando não há sítios reprodutivos nas imediações. As espécies que evitam a matriz, como os anfíbios com desenvolvimento direto dos girinos, certamente sofrem em longo prazo com problemas genéticos devido à baixa contribuição demográfica de imigrantes ou através de extinções locais estocásticas. Contudo, os impactos das forçadas migrações reprodutivas através da matriz devem resultar em declínio das populações de espécies com desenvolvimento larval aquático em prazos muito mais breves. É preciso considerar a importância das matas de galeria e o fortalecimento das leis que as protegem para que estratégias conservacionistas sejam efetivas na preservação dos recursos hídricos para os humanos e para uma classe de vertebrados tão ameaçada e importante para o funcionamento dos ecossistemas como os anfíbios.



Representação da desconexão entre fragmentos florestais e os riachos de São Luis do Paraitinga-SP a lápis preto / por Débora Becker.