UNIVERSIDADE DO VALE DO RIO DOS SINOS – UNISINOS
UNIDADE ACADÊMICA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA
NÍVEL DOUTORADO

LEONARDO FELIPE BAIROS MOREIRA

CONSERVAÇÃO DE ANFÍBIOS EM ÁREAS AGRÍCOLAS DO SUL DO BRASIL

SÃO LEOPOLDO

2013

LEONARDO FELIPE BAIROS MOREIRA

CONSERVAÇÃO DE ANFÍBIOS EM ÁREAS AGRÍCOLAS DO SUL DO BRASIL

Tese apresentada como requisito parcial para a obtenção do título de Doutor, pelo Programa de Pós-Graduação em Biologia da Universidade do Vale do Rio dos Sinos – UNISINOS.
Área de concentração: Diversidade e Manejo de Vida Silvestre

Orientador: Leonardo Maltchik Garcia

SÃO LEOPOLDO

2013

LEONARDO FELIPE BAIROS MOREIRA

CONSERVAÇÃO DE ANFÍBIOS EM ÁREAS AGRÍCOLAS DO SUL DO BRASIL

Tese apresentada como requisito parcial para a obtenção do título de Doutor, pelo Programa de Pós-Graduação em Biologia da Universidade do Vale do Rio dos Sinos – UNISINOS.

Aprovado em

BANCA EXAMINADORA

Dr. Leonardo Maltchik Garcia - Universidade do Vale do Rio dos Sinos

Dr. Fausto Nomura - Universidade Federal de Goiás

Dr. Márcio Borges Martins - Universidade Federal do Rio Grande do Sul

Dr. Mirco Solé - Universidade Estadual de Santa Cruz

Dr. Patrick Colombo - Universidade Federal do Rio Grande

Dedico essa tese aos meus pais e
aos bons professores que me inspiraram
de uma forma muito positiva. Este
trabalho tem um pouco de cada um deles.

AGRADECIMENTOS

Aos meus pais e familiares pelo apoio e por tolerarem minhas esquisitices criando anfíbios em aquários.

A todos os membros da banca pela participação.

Agradeço a todos que de alguma forma me ajudaram a realizar este trabalho, e deixo um pedido de desculpas, inclusive, aos que eu, por acaso, tenha esquecido de citar.

<div align="center">OBRIGADO!</div>

RESUMO

Iniciativas de conservação em paisagens agrícolas têm se tornado uma das prioridades na agenda de conservação de anfíbios, já que mudanças no uso da terra estão associadas com declínios globais. Nesta tese, eu procurei analisar os efeitos da fragmentação e intensificação agrícola na riqueza e composição de anfíbios anuros, em áreas úmidas do bioma Pampa no Rio Grande do Sul. A tese está dividida em três capítulos. No primeiro, eu avaliei diferenças em assembleias de anuros entre áreas naturais, arrozais orgânicos e arrozais não orgânicos. No segundo capítulo, eu investiguei os padrões de coocorrência e aninhamento em assembleias associadas à áreas naturais e arrozais com diferentes idades de cultivo. No último capítulo, eu examinei a relação entre abundância de girinos e características do habitat em cinco escalas espaciais, na metade sul do Rio Grande do Sul. Considerando os três estudos, é possível concluir que ações de conservação focadas em anfíbios que utilizam áreas agrícolas requerem o manejo de diferentes elementos em múltiplas escalas espaciais. Diferentes espécies de anuros não respondem à mesma escala espacial ou a diferenças históricas entre as áreas. Portanto, determinar a extensão das respostas espécie-específicas é uma etapa urgente para alternativas de conservação viáveis, em paisagens agrícolas do bioma Pampa.

Palavras-chave: Áreas campestres. Agroecossistemas. Paisagem. Histórico.

ABSTRACT

Conservation initiatives in agricultural landscapes have become one of the priorities on the conservation agenda of amphibians, because land use changes are associated with global declines. In this thesis, I tried to analyze the effects of agricultural fragmentation and intensification on anuran species richness and composition, in wetlands of Pampa biome in Rio Grande do Sul. The thesis has three chapters. In the first one, I evaluated differences in anuran assemblages across natural areas, organic rice fields, and non-organic rice fields. In the second chapter, I investigated species co-occurrence and nestedness patterns in assemblages associated to natural areas and rice fields with different crop ages. In the last chapter, I examined the relationship between tadpole abundance and habitat features at 5 spatial scales, in southern half of Rio Grande do Sul state. In general, I conclude that amphibian conservation actions in agricultural areas need the management of different elements at multiple spatial scales. Different anuran species do not all respond to the same spatial scale or to historic differences across the areas. Therefore, to determine the extent of species-specific responses are an urgent steep toward workable conservation alternatives across agricultural landscapes in Pampa biome.

Keywords: Grasslands. Agroecosystems. Landscape. Historic.

SUMÁRIO

1 INTRODUÇÃO GERAL

No Brasil, o bioma Pampa abrange os campos da região das Missões e da metade sul do Rio Grande do Sul, com uma área de aproximadamente 178.243 km^2 (IBGE, 2004). O Pampa brasileiro é uma porção dos Pampas Sul-Americanos, um mosaico de vegetações campestres e arbustivas, que se estendem pelos territórios da Argentina, Brasil, Paraguai e Uruguai (MIÑARRO; BILLENCA, 2008). De acordo com a classificação da *WWF Conservation Science* (OLSON et al., 2001), a parte brasileira do bioma está dentro da ecorregião das savanas uruguaias (NT 0710), possuindo um relevo relativamente plano, com elevações que vão desde o nível do mar até altitudes de 500 m em algumas áreas. A maior parte da região inclui campos de meia altura com formações florestais pouco expressivas e frequentemente associadas às margens de rios (BOLDRINI, 2009). Apesar da aparente homogeneidade estrutural da vegetação, o Pampa abriga uma rica diversidade faunística, que inclui espécies endêmicas e ameaçadas de extinção (BENCKE, 2009).

As savanas uruguaias são consideradas criticamente ameaçadas com poucos remanescentes isolados de fragmentos não modificados (CORDEIRO; HASENACK, 2009). Toda a região foi severamente alterada pela criação de gado, introduzido na região ainda no século XVII (CRAWSHAW et al., 2007). A crescente expansão agrícola e o incentivo da silvicultura também têm contribuído para esse cenário. Somente nos últimos 30 anos, houve uma redução de 25 % nas áreas de campos devido a forte expansão agrícola, principalmente das lavouras de arroz, milho e soja (OVERBECK et al., 2007). O cultivo de árvores exóticas tem sido incentivado por políticas públicas no sul do Brasil, principalmente para a produção de celulose. Monoculturas florestais exóticas geram inúmeros impactos sobre a flora e fauna nativas, pois acarretam grandes mudanças nas condições de luz, regime hídrico, estrutura de habitat e disponibilidade de alimento (BINKOWSKI, 2009; MACHADO; MOREIRA; MALTCHIK, 2012; OVERBECK et al., 2007).

Mesmo sendo um ecossistema típico do sul do Brasil, o grau de conservação do Pampa é muito baixo. Menos de 0,5% das áreas campestres do Rio Grande do Sul estão atualmente inseridas em áreas de proteção integral (OVERBECK et al., 2007; PILLAR; VÉLEZ, 2010). Mesmo unidades de conservação, como a Reserva Biológica do Ibirapuitã e o Parque Estadual do Espinilho, apresentam algum tipo de pressão antrópica, especialmente a pecuária. Nesta realidade, onde a maior parte do bioma está em áreas privadas com uso agropastoril, o desenvolvimento de conceitos e práticas de manejo que ajudem a manter a biodiversidade nessas áreas é essencial.

Desde a década de 1990, inúmeros estudos ao redor do mundo têm alertado a respeito de declínios populacionais em espécies de anfíbios. Apesar do aumento da consciência pública sobre a importância das populações de anfíbios remanescentes e dos fatores responsáveis pelo seu desaparecimento (BECKER; ZAMUDIO, 2011; BLAUSTEIN; WAKE, 1990; BLAUSTEIN et al., 2010; ETEROVICK et al., 2005), existe uma discrepância sobre declínios e expansões populacionais em países megadiversos (PIMENTA et al., 2005; STUART et al., 2004). Atualmente o Brasil conta com 946 espécies de anfíbios (SEGALLA et al., 2012), das quais 39 estão em alguma categoria de ameaça em curto prazo (SUBIRÁ et al., 2012). No entanto, há um elevado número de espécies incluídas na categoria "dados insuficientes" (151 spp.) ou cuja situação não foi avaliada (67 spp.). Essa informação aliada ao fato de que praticamente não há monitoramentos populacionais de anfíbios em larga escala no Brasil revela o precário conhecimento existente sobre essa fauna.

Embora as razões para o declínio dos anfíbios possam variar de região para região e mesmo dentro de diferentes populações da mesma espécie (BLAUSTEIN et al., 2010), atualmente há um consenso de que declínios, em espécies que ocorrem em baixas altitudes, estão mais frequentemente associados à perda ou modificação do habitat (BECKER; ZAMUDIO, 2011; CUSHMAN, 2006; GALLANT et al., 2007; HERO; MORRISON, 2004). Outros fatores aparentemente sérios são as infecções causadas pelo fungo *Batrachochytrium dendrobatidis* e a introdução de espécies exóticas, como a rã-touro (*Lithobates catesbeianus*) (BECKER; ZAMUDIO, 2011; BOTH et al., 2011).

A agricultura ocupa uma porção de terra muito maior do que qualquer outra atividade humana (DEVINE; FURLONG, 2007), com previsões de aumento em função da demanda por produtos alimentícios e biocombustíveis (FERREIRA et al., 2012; SPAROVEK et al., 2010). Assim a perda de habitat em função da conversão para agricultura é, provavelmente, o mais importante fator que afeta as populações de anfíbios. Impactos diretos da expansão agrícola estão relacionados ao aumento da fragmentação e desconexão de habitats (BECKER et al., 2007; FICETOLA; DE BERNARDI, 2004), enquanto que a intensificação da produção afeta a qualidade e a disponibilidade de habitats para forrageio e reprodução (PIHA; LUOTO; MERILA, 2007). Além dos fatores acima mencionados, estudos começaram a demonstrar evidências de efeitos sinergéticos entre dois ou mais fatores associados, potencializando os efeitos de atividades agrícolas nos declínios de anfíbios (MANN et al., 2009).

A interpretação dos efeitos da expansão agrícola é claramente dependente da escala investigada e da tolerância do organismo investigado frente a essas modificações (BENTON; VICKERY; WILSON, 2003; DIXO; METZGER, 2010; ERNST; RÖDEL, 2008). Devido ao

seu ciclo de vida bifásico, espécies de anfíbios com larvas aquáticas ocupam distintos habitats que incluem ambientes aquáticos para os girinos, ambientes terrestres para forrageio de juvenis e adultos e uma matriz que permita o deslocamento entre esses ambientes (BECKER et al., 2007; SEMLITSCH, 2008). Portanto, é provável que diferentes processos da intensificação agrícola tenham influências diferenciadas nas fases aquática e terrestre. Outro ponto importante é que a distribuição das espécies em áreas com grande fragmentação é influenciada não somente por características atuais da paisagem, mas também pelo seu histórico (LUNT; SPOONER, 2005; PIHA; LUOTO; MERILA, 2007). Em áreas com longo histórico agrícola é esperada uma redução da qualidade do habitat, devido ao acúmulo de pesticidas no sedimento e menor heterogeneidade da paisagem.

O cultivo do arroz ocupa cerca de 11% das terras aráveis do mundo e a conversão para sua produção tem sido apontada como uma das principais causas da perda de áreas úmidas (DONALD, 2004). Em muitos locais, as áreas úmidas naturais foram completamente substituídas por campos de arroz e populações da fauna nativa dependem fortemente desses agroecossistemas (FUJIOKA; LANE, 1997; REID; HEITMEYER, 1995). No entanto, a utilização dos arrozais como substitutos de áreas úmidas naturais pode variar em função do método de produção, tipo de plantio, irrigação e manejo na entressafra. Técnicas de manejo conhecidas por afetarem o uso dos arrozais incluem o manejo orgânico ou com agroquímicos, controle da água de irrigação e no período de entressafra e o manejo das populações de peixes (DONALD, 2004; KATO et al., 2010; MACHADO; MALTCHIK, 2010; WILSON; WATTS; STEVENS, 2007). O estado do Rio Grande do Sul é o maior produtor nacional de arroz, respondendo por 67,5 % da produção desse cereal (IBGE, 2013). As principais áreas orizícolas estão na metade sul do estado, onde se concentram as principais áreas úmidas do estado (MALTCHIK et al., 2003).

Nesse contexto, o objetivo geral dessa tese foi analisar os efeitos da fragmentação e intensificação agrícola na riqueza e composição de anfíbios anuros, em áreas úmidas continentais do Rio Grande do Sul, na ecorregião das savanas uruguaias. A tese está estruturada em três capítulos. O primeiro capítulo, "*Does organic agriculture benefit anuran diversity in rice fields*?" é um manuscrito que foi submetido para a revista *Biodiversity and Conservation*. O objetivo principal desse capítulo foi comparar as assembleias de anuros em arrozais cultivados sobre diferentes regimes de manejo comerciais, enfocando três aspectos. Primeiro, nós avaliamos se arrozais orgânicos diferem dos não orgânicos com relação à composição, riqueza e diversidade de espécies. Segundo, nós comparamos diversidade de

anuros e composição entre arrozais e áreas úmidas naturais. Por último, descrevemos mudanças sazonais nas assembleias ao longo do ciclo de cultivo.

No segundo capítulo desta tese, nós examinamos os padrões de coocorrência e aninhamento em assembleias de anuros associadas às áreas naturais e arrozais com diferentes idades de cultivo. Nossas hipóteses foram: i) assembleias de anuros em arrozais mostrariam aninhamentos significativos e esse padrão seria guiado pelo histórico da área (sugerindo a predominância de dinâmicas de extinção-colonização); ii) guildas de anuros seriam afetadas de forma distinta pela idade da lavoura, como resultados de suas habilidades de dispersar através da matriz agrícola. Assim espécies terrestres podem ficar confinadas dentro das áreas cultivadas, espécies aquáticas irão dispersar somente durante o período de crescimento do arroz e espécies empoleiradoras não ficariam confinadas dentro das áreas cultivadas. A partir dos resultados do segundo capítulo foi redigido o manuscrito "*Amphibians in an agricultural area: the community structure is related to crop age*" submetido ao periódico *Acta Oecologica*.

O terceiro e último capítulo, "*Anuran abundance in the Pampa wetlands: the role of local and landscape factors*" será submetido para a revista *Landscape Ecology*. Nesse trabalho nós examinamos a relação entre a abundância de anuros e características do habitat no bioma Pampa, em cinco escalas espaciais. Nossos objetivos foram: i) determinar a abundância de três espécies de anuros em 30 áreas úmidas sujeitas à fragmentação agrícola e pecuária; ii) determinar em qual escala espacial as espécies respondem as modificações do habitat; iii) examinar as relações entre abundância de anuros e características dos habitats.

1.1 REFERÊNCIAS

BECKER, C. G. et al. Habitat split and the global decline of amphibians. **Science**, v. 318, n. 5857, p. 1775-1777, 2007.

BECKER, C. G.; ZAMUDIO, K. R. Tropical amphibian populations experience higher disease risk in natural habitats. **Proceedings of the National Academy of Sciences of the United States of America**, v. 108, n. 24, p. 9893-9898, 2011.

BENCKE, G. A. Diversidade e conservação da fauna dos campos do sul do Brasil. In: PILLAR, V. D. et al. (Org.). **Campos sulinos: conservação e uso sustentável da biodiversidade.** Brasília: Ministério do Meio Ambiente, 2009. p. 101-121.

BENTON, T. G.; VICKERY, J. A.; WILSON, J. D. Farmland biodiversity: is habitat heterogeneity the key? **Trends in Ecology & Evolution**, v. 18, n. 4, p. 182-188, 2003.

BINKOWSKI, P. **Conflitos ambientais e significados sociais em torno da expansão da silvicultura de eucalipto na "Metade Sul" do Rio Grande do Sul**. Dissertação (Mestrado em Desenvolvimento Rural) -- Programa de Pós Graduação em Desenvolvimento Rural Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 2009.

BLAUSTEIN, A. R. et al. Direct and indirect effects of climate change on amphibian populations. **Diversity**, v. 2, n. 2, p. 281-313, 2010.

BLAUSTEIN, A. R.; WAKE, D. B. Declining amphibian populations: a global phenomenon? **Trends in Ecology & Evolution**, v. 5, p. 203-204, 1990.

BOLDRINI, I. A flora dos campos do Rio Grande do Sul. In: PILLAR, V. D. et al. (Org.). **Campos sulinos: conservação e uso sustentável da biodiversidade.** Brasília: Ministério do Meio Ambiente, 2009. p. 63-77.

BOTH, C. et al. Widespread occurrence of the American Bullfrog , *Lithobates catesbeianus* (Shaw , 1802) (Anura: Ranidae), in Brazil. **South American Journal of Herpetology**, v. 6, n. 2, p. 127-134, 2011.

CORDEIRO, J. L. P.; HASENACK, H. Cobertura vegetal atual do Rio Grande do Sul. In: PILLAR, V. D. et al. (Org.). **Campos sulinos: conservação e uso sustentável da biodiversidade.** Brasília: Ministério do Meio Ambiente, 2009. p. 285-299.

CRAWSHAW, D. et al. Caracterização dos campos sul-rio-grandenses: uma perspectiva da ecologia da paisagem. **Boletim Gaúcho de Geografia**, v. 33, p. 233-252, 2007.

CUSHMAN, S. A. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. **Biological Conservation**, v. 128, n. 2, p. 231-240, 2006.

DEVINE, G. J.; FURLONG, M. J. Insecticide use: contexts and ecological consequences. **Agriculture and Human Values**, v. 24, p. 281-306, 2007.

DIXO, M.; METZGER, J. P. The matrix-tolerance hypothesis: an empirical test with frogs in the Atlantic Forest. **Biodiversity and Conservation**, v. 19, n. 11, p. 3059-3071, 2010.

DONALD, P. F. Biodiversity impacts of some agricultural. **Conservation Biology**, v. 18, n. 1, p. 17-37, 2004.

ERNST, R.; RÖDEL, M. O. Patterns of community composition in two tropical tree frog assemblages: separating spatial structure and environmental effects in disturbed and undisturbed forests. **Journal of Tropical Ecology**, v. 24, n. 02, p. 111-120, 2008.

ETEROVICK, P. C. et al. Amphibian Declines in Brazil: an Overview. **Biotriopica**, v. 37, n. 2, p. 166-179, 2005.

FERREIRA, J. et al. Towards environmentally sustainable agriculture in Brazil: challenges and opportunities for applied ecological research. **Journal of Applied Ecology**, v. 49, p. 535-541, 2012.

FICETOLA, G. F.; DE BERNARDI, F. Amphibians in a human-dominated landscape: the community structure is related to habitat features and isolation. **Biological Conservation**, v. 119, n. 2, p. 219-230, 2004.

FUJIOKA, M.; LANE, S. J. The impact of changing irrigation practices in rice fields on frog populations of the Kanto Plain, central Japan. **Ecological Research**, v. 12, n. 1, p. 101-108, 1997.

GALLANT, A. L. et al. Global rates of habitat loss and implications for amphibian conservation. **Copeia**, p. 967-979, 2007.

HERO, J. M.; MORRISON, C. Frog declines in Australia: global implications. **Herpetological Journal**, v. 14, p. 175-186, 2004.

IBGE. **Mapa de biomas do Brasil**. Disponível em: < http://www.ibge.gov.br >. Acesso em: 23 abr. 2013.

IBGE. **Levantamento sistemático da produção agrícola**. Rio de Janeiro: 2013. Disponível em: <http://www.ibge.gov.br/home/estatistica/indicadores/agropecuaria/lspa/estProdAgr_201303.pdf>. Acesso em: 23. abr. 2013.

KATO, N. et al. Differential responses of two anuran species breeding in rice fields to landscape composition and spatial scale. **Wetlands**, v. 30, n. 6, p. 1171-1179, 2010.

LUNT, I. D.; SPOONER, P. G. Using historical ecology to understand patterns of biodiversity in fragmented agricultural landscapes. **Journal of Biogeography**, v. 32, n. 11, p. 1859-1873, 2005.

MACHADO, I. F.; MALTCHIK, L. Can management practices in rice fields contribute to amphibian conservation in southern Brazilian wetlands? **Aquatic Conservation: Marine and Freshwater Ecosystems**, v. 46, n. 950, p. 39-46, 2010.

MACHADO, I. F.; MOREIRA, L. F. B.; MALTCHIK, L. Effects of pine invasion on anurans assemblage in southern Brazil coastal ponds. **Amphibia-Reptilia**, v. 33, n. 2, p. 227-237, 2012.

MALTCHIK, L. et al. Inventory of wetlands of Rio Grande do Sul (Brazil). **Pesquisas Botânica**, v. 53, p. 89-100, 2003.

MANN, R. M. et al. Amphibians and agricultural chemicals: review of the risks in a complex environment. **Environmental pollution**, v. 157, n. 11, p. 2903-2927, 2009.

MIÑARRO, F.; BILLENCA, D. **The conservation status of temperate grasslands in central Argentina**. Buenos Aires, Argentina: Fundación Vida Silvestre Argentina, 2008.

OLSON, D. M. et al. Terrestrial ecoregions of the world: a new map of life on Earth. **BioScience**, v. 51, n. 11, p. 933-938, 2001.

OVERBECK, G. et al. Brazil's neglected biome: the South Brazilian Campos. **Perspectives in Plant Ecology, Evolution and Systematics**, v. 9, n. 2, p. 101-116, 2007.

PIHA, H.; LUOTO, M.; MERILA, J. Amphibian occurrence is influenced by current and historic landscape characteristics. **Ecological Applications**, v. 17, n. 8, p. 2298-2309, 2007.

PILLAR, V. D. P.; VÉLEZ, E. Extinção dos campos sulinos em unidades de conservação: um fenômeno natural ou um problema ético? **Natureza & Conservação**, v. 08, n. 01, p. 84-86, 2010.

PIMENTA, B. V. S. et al. Comment on "Status and trends of amphibian declines and extinctions worldwide". **Science**, v. 309, p. 1999, 2005.

REID, F. A.; HEITMEYER, M. E. Waterfowl and rice in California's Central Valley. **California Agriculture**, v. 49, p. 62, 1995.

SEGALLA, M. V. et al. **Brazilian amphibians – List of species**. Disponível em: <www.sbherpetologia.org.br>. Acesso em: 8 abr. 2013.

SEMLITSCH, R. D. Differentiating migration and dispersal processes for pond-breeding amphibians. **Journal of Wildlife Management**, v. 72, n. 1, p. 260-267, 2008.

SPAROVEK, G. et al. Brazilian agriculture and environmental legislation: status and future challenges. **Environmental Science & Technology**, v. 44, n. 16, p. 6046-53, 2010.

STUART, S. N. et al. Status and trends of amphibian declines and extinctions worldwide. **Science**, v. 306, p. 1783-1786, 2004.

SUBIRÁ, R. J. et al. Avaliação científica do risco de extinção da fauna brasileira – resultados alcançados em 2012. **Biodiversidade Brasileira**, v. 2, n. 2, p. 124-130, 2012.

WILSON, A. L.; WATTS, R. J.; STEVENS, M. M. Effects of different management regimes on aquatic macroinvertebrate diversity in Australian rice fields. **Ecological Research**, v. 23, n. 3, p. 565-572, 2007.

2 CAPÍTULO 1

Este capítulo foi submetido na forma de artigo científico ao periódico *Biodiversity and Conservation*.

Does organic agriculture benefit anuran diversity in rice fields?

Leonardo Felipe Bairos Moreira[1,*] and Leonardo Maltchik[1]

[1] Laboratório de Ecologia e Conservação de Ecossistemas Aquáticos, Universidade do Vale do Rio dos Sinos-UNISINOS, Av. Unisinos, 950, 93022-000, São Leopoldo, Brasil

* corresponding author: e-mail: leonardobm@gmail.com ; phone number: 55-51-3591-1100 ; fax number: 55-51-3590-8122

Abstract

The debate about management practices that help maintain biodiversity in cultivated areas is an ongoing controversy in conservation biology. The theory suggests that organic agriculture supports greater levels of diversity than non-organic. This study examined anuran assemblages in natural intermittent ponds and rice fields under two types of commercial management (conventional and organic) in southern Brazil. We tested the differences in species composition, richness and diversity among assemblages and ecomorphological guilds, at different stages of rice cultivation. Overall, organic fields had a greater diversity of anurans than the non-organic fields, and they had a different species composition. Most of the significant differences observed between the natural areas and the rice fields occurred during the off-season. Aquatic and perches species were abundant in natural areas. Perches and terrestrial species were associated with organic rice fields, while terrestrial species were more common in conventional rice fields. We observed that the effect of management regime could be affected by a combination between the existence of barriers and species dispersal ability. Thus, the incorporation of individual traits of each species (e.g., habitat preference and reproductive mode) is fundamental to the creation of more effective conservation strategies in agroecosystems.

Keywords: amphibian conservation; community composition; guilds, agroecosystems; southern Brazil

2.1 INTRODUCTION

Agriculture occupies a larger portion of land than any other human activity (Devine and Furlong 2007), and its expansion and intensification affect biodiversity (Gallant et al. 2007; Gray et al. 2004). While increased fragmentation and habitat split are direct impacts of agricultural expansion (Cosentino et al. 2010; Ficetola and De Bernardi 2004), intensification directly affects the quality and availability of habitat for foraging and reproduction (Benton et al. 2003; Piha 2006). Organisms that depend on wetlands surrounded by agricultural fields are exposed to high levels of agrochemicals, which may affect the biota in an isolated manner or in combination with other factors (Mann et al. 2009; Peltzer et al. 2008). Therefore, it is essential to understand the biology and dynamics of communities that use cultivated areas and develop concepts and management practices that help maintain biodiversity in these areas.

The use of organic techniques is currently an alternative method to conventional farm management. Studies indicate that organic agriculture supports greater levels of biodiversity than non-organic management methods (Fuller et al. 2005; Hole et al. 2005). These benefits are attributed to intrinsic, but not exclusive, practices of organic systems, such as no or little use of chemical pesticides and inorganic fertilizers, crop rotation and maintenance of a heterogeneous landscape around crop edges. Overall, organic crops differ from non-organic crops in the size of area cultivated, composition and management (Fuller et al. 2005). However, reviews about the theme suggest that the biodiversity response to this management varies across crops and taxonomic groups studied (Andersen and Eltun 2000; Beecher et al. 2002; Fuller et al. 2005; Weibull et al. 2000).

Rice (*Oryza* spp.) is the most common cultivated cereal in the world and occupies close to 11% of the world's arable land (Donald 2004). In many regions, rice fields are considered important substitutes of wetlands and many organisms use these cultivated fields as areas for foraging and reproduction (Duré et al. 2008; Machado and Maltchik 2010; Mann et al. 2009; Piatti et al. 2010; Wilson et al. 2007). According to the type of management employed, rice production could create a mosaic of microhabitats that are favorable to different groups of fauna and flora, at a proportion similar to what is found in natural areas (Bambaradeniya et al. 2004; Duré et al. 2008; Wilson et al. 2007).

Among the groups of vertebrates, amphibians have the greatest proportion of threatened species (Beebee and Griffiths 2005), and fragmentation and habitat split have led to these species becoming threatened (Becker et al. 2007). Various amphibian species have occupied dams and irrigation canals since they are able to colonize these areas by terrestrial dispersal (Marsh et al. 2004; Vasconcelos and Calhoun 2004), and in the absence of less mobile predators such as fish, amphibians have persisted as metapopulations in agricultural areas (Herzon and Helenius 2008; Knutson et al. 2004). However, one of the difficulties of managing modified landscapes is that species' susceptibility to fragmentation is related to individual characteristics of each species and partially depends on dispersal ability, reproductive modes and habitat preference (Cushman 2006; Dixo and Metzger 2010). Furthermore, there are gaps in our knowledge of the basic biology of many species and how it is related with habitat loss and fragmentation (Cushman 2006; Hazell 2003).

When data about basic biology are scarce, an alternative is to use the guild approach. A guild includes species that share ecological similarities independent of phylogenetic similarity (Blondel 2003). Recent studies on anuran communities in agricultural environments found conflicting results between pre-defined groups of species (Becker et al. 2007; Dixo and Metzger 2010; Peltzer et al. 2008). Although the relationship between amphibians and rice fields has been investigated in areas with high production (e.g., Bambaradeniya et al. 2004; Fujioka and Lane 1997; Kato et al. 2010), studies about this subject are rare and recent in South America (Duré et al. 2008; Machado and Maltchik 2010; Piatti et al. 2010). Information about the role of these agroecosystems in amphibian conservation is important in southern Brazil since the region contains around 10% of the amphibian species of Brazil (Segalla et al. 2012), which is the country with the greatest amphibian diversity in the world and the ninth greatest rice production (FAO Stat. 2010).

We compared anuran assemblages in rice fields cultivated under different commercial management regimes, used in southern Brazil, focusing on three aspects: 1) We evaluated whether organic rice fields had a different species composition, richness and diversity than non-organic fields; 2) We compared anuran diversity and composition between rice fields and natural ponds; and 3) We described seasonal differences in assemblages throughout the crop cycle. Considering a decreasing gradient of disturbance (conventional crop → organic crop →natural ponds), our hypothesis is that the

anuran guilds are distinctly affected by different agricultural management regimes. Thus, perches species will show higher number of species, diversity and abundance in organic crops in relation to conventional crop. Aquatic species will be abundant only during rice growing period. Terrestrial species, due to calling close to the ground, will not be affect by rice field management.

2.2 MATERIALS AND METHODS

Rio Grande do Sul is responsible for 67.5% of the rice production in Brazil, and the coastal plain is an important area of rice production in South America (Azambuja et al. 2004). The study took place from August 2011 to August 2012 in an agricultural area dedicated to irrigated rice production (30.705° to 30.755°S ; 51.630° to 51.700°W). The area is located in Sentinela do Sul, in the central-west portion of the coastal plain (Fig.1). The climate is subtropical, moderately humid, and the temperature varies between 11°C in the winter and 26°C in the summer, with an average annual temperature of 18.5°C. Annual precipitation varies from 1500 to 1700 mm/year (Rossato 2011).

The rice plantations at study area are divided in various 1 ha plots that are interconnected by secondary roads and drainage canals. These drainage canals (2-5 m wide and 0.5-1.5 m deep) are filled by water from nearby streams, which provide water for the cultivated plots (~ 10 cm water per 130 days) during the rice cultivation cycle. The rice fields were divided into two types according the management regimes used.

1. Conventional: Sowing is manual or with the aid of machinery. As soon as seedlings are established (5-10 cm tall), the fields are permanently flooded. Application of artificial fertilizers, insecticides and herbicides is concentrated in the initial growth stage.

2. Biodynamic: Agrochemicals are not applied to the organic fields at any stage of production. Organic rice fields are planted with the aid of light machinery, which provides some protection against pests. A rotation in the flooding regime is conducted to eliminate unwanted species and keep the soil fertile.

Three replicates of rice plots in each of the different management regimes were sampled within a 10 km radius (Fig. 1). We avoided using parcels near other types of vegetation because we were interested in species associated with rice fields. We only investigated parcels surrounded by

other parcels of rice in the conventional crops. This was not possible in the organic crops because of the practices employed in the area which support crops to grow next to an edge of native vegetation. We sampled three ponds to compare the anuran distribution and abundance between native areas and areas modified by rice production. We tried to select areas that were similar in size to the rice fields and had temporary hydroperiods that lasted at least five months. The selected areas were at least 600 m from one another to minimize spatial autocorrelation. The spatial independence of the nine sampling areas was tested using PCNM analysis. As the PCNM had not detected significant spatial structure ($P = 0.86$), it was not included in the statistical analysis.

Sampling occurred five times during the rice cultivation cycle: two samples in the off-season period (August/2011 and August 2012), two samples during the growing period (January/2012 and March/2012) and one sample during the post-harvest period (June/2012). Anuran richness and abundance was measured using visual and acoustic searches (Crump and Scott 1994). The sampling occurred between 19:00h and 24:00h, and the areas were sampled in a random order. In each sample, we followed a 100 m transect perpendicular to the rice fields and ponds for 20 minutes. All of the individuals located visually or acoustically were registered. Pilot studies showed that 20 minutes was sufficient to sample an entire rice field parcel. The species were separated in guilds according to the criteria described by Vallan (2000): (aquatic) found floating at the water surface; (terrestrial) found on the ground; (perches) found in herbaceous habitats and bushes. We calculated the diversity index for each area, in each cultivation period, based on the Rényi-generalized entropy formula (Anand and Orlóci 1996). We adopted the entropy order 12, which stabilizes the effect of equitability (Duarte et al. 2006).

We compared the differences in richness, abundance and diversity of anurans among the different areas (conventional rice field, organic rice field and natural pond) throughout the cultivation cycle with an ANOVA. Since temporal differences in the association of assemblages with the cultivation cycle were anticipated (Machado and Maltchik 2010), we also investigated the effect of the interaction between the crop cycle and type of management of the areas. When the variance analysis indicated significant differences between the groups, we conducted a Tukey test to verify which groups differed from one another (Zahr 1999). The analyses were conducted for the whole

assemblages and for each guild. Abundance and richness were transformed to the squared root to reduce heteroscedasticity. Environment R 2.15.0 (R Development Core Team 2012) was used to conduct the analyses.

We used non-metric multidimensional scaling (NMDS) to evaluate the differences in the anuran composition between natural areas and areas that have been converted to rice fields. The similarity matrices were calculated using Bray-Curtis distance and scaling directed to two axes. A permutational variance analysis (PERMANOVA) was used to test the significance of any difference in composition observed by the NMDS. Differences between the management regimes were also analyzed separately between the periods of off-season, growing and post-harvest. The similarity matrices were calculated using a Bray-Curtis distance and 1000 permutations were run to validate the results. The analyses were conducted on the vegan package 2.0.3 for R (Oksanen et al. 2012). Additional information was obtained from the species indicator analysis (Indval) (Dufrêne and Legendre 1997), to ascertain which species could serve as indicators in different rice management regimes. The matrices were constructed considering the composition in the different cultivation periods and the analyses were conducted with the package labdsv 1.5.0 for R (Roberts 2012).

2.3 RESULTS

We registered a total of 17 species, distributed in five families (Table 1). Of these species found, 10 were classified as terrestrial. The group of perches anurans was composed of all of the representatives of the family Hylidae, with the exception of *Pseudis minuta*. Since it was the only species with predominantly aquatic habits in the study area, *P. minuta* was only included in the analyses that considered all of the species. Although the abundance and richness did not differ between the management regimes (Table 2), organic rice fields had a higher mean diversity of species than conventional crops (Tukey, p = 0.04). Mean diversity was 2.2 times higher in organic crops. There was no interaction with the cultivation period for any of the rice field comparisons, which indicates that this response pattern was not associated with the seasonal availability of water in the fields.

Mean overall, richness, abundance and diversity were greater in natural areas. However, there was an interaction with the cultivation period in the comparisons between ponds and rice fields (Table 2). In the off-season periods, the ponds had significantly higher abundance than either of the rice fields (Fig. 2). Perches species showed a tendency that was similar to the general community. Terrestrial species abundance did not differ between rice fields and ponds, although the interaction with the cultivation period was significant. At the end of the growing period, terrestrial anuran abundance was significantly higher in ponds than in cultivated areas (Fig. 2).

Richness was lower in organic rice fields than in natural areas at the late growing period (Fig. 3). Richness was lower in conventional rice fields in the off-season and growing periods. Perches species richness was the greatest in ponds and did not interact with cultivation period (Table 2). Terrestrial species richness did not differ between rice fields and natural areas, although it interacted significantly with cultivation period. As was seen with abundance, richness was greatest in ponds at the end of the growing period (Fig. 3). The differences in diversity were less evident. With relation to the whole community, the natural areas were more diverse than the conventional rice fields only in the off-season period (Fig. 4). Perches species diversity was significantly different between ponds and both types of management during the off-season periods (Table 2). Terrestrial species diversity was higher in organic rice fields at the beginning of the growing season (Fig. 4).

In the NMDS ordination (Fig. 5), rice fields and natural areas formed well-defined groups. The anuran community composition was similar in conventional and organic crops. Aquatic and perches species were abundant in ponds. Perches and terrestrial species were associated with organic rice fields, and terrestrial species were more common in conventional rice fields. The PERMANOVA, which included the entire cultivation cycle, showed significant differences between the communities of organic rice fields, conventional rice fields and ponds (F= 3.43, gl= 2, p = 0.034). Comparison of the assemblages in the crop cycle showed that there were significant differences only during the off-season period (Table 3).

We identified seven indicator species associated with ponds and conventional rice fields (Table 04), and more species were found in natural areas (06 species). Representatives of the family

Hylidae were responsible for all of the indicator species during the off-season period, while terrestrial species (Leiuperidae and Leptodactylidae) were associated with the rice growing period.

2.4 DISCUSSION

Our results indicate that organic crops host a greater diversity of anurans and have a different community composition than non-organic crops. Although this pattern is consistent with other previous studies (Fuller et al. 2005; Hole et al. 2005), the idea that organic agriculture favors diversity is not necessarily true. Since organic crops have less area and greater edges with non-cultivated area, the effects of crop management could be confused with the variability of the habitat. In some cases, the landscape structure seems to be more important to species diversity and composition than the type of agricultural management used (Weibull et al. 2000). Temporal scale could also obscure the effects of organic agriculture. Organic crops affect important factors for biodiversity and the beneficial effects on diversity may take time to manifest (Anderson et al. 2010; Rundlo et al. 2011). The duration of this time lag will depend on multiple factors such as the presence of source areas for species recolonization, vegetation succession and biotic interactions. In the study area, the organic crops are cultivated for up to 25 years. Assuming that the species of the community have a generation time of three years, anurans living in these rice fields are subject to management for only eight generations. Furthermore, organic rice fields did not differ from the conventional fields when the analyses were focused on guilds. Thus, it is reasonable to assume that differences related to species groups, which have different life histories, and habitat variability are responsible for the differences in diversity between natural ponds and rice fields. In species composition pattern analyses, treating distinct groups of anurans as one entity could obscure or confuse group-specific patterns (Ernst and Rödel 2008).

Species composition varied considerably between natural ponds and rice fields. We noted reduced anuran abundance in cultivated areas and a marked interaction with the period of cultivation. Changes during cultivation include changes in the water depth and structural diversity of the vegetation. Most of the significant differences that occurred between natural areas and rice fields occurred during the off-season period. These results were a reflection of the greater abundance of treefrogs in natural areas that calling from floating vegetation (e. g.: *Dendropsophus minutus*, *Pseudis*

minuta) or herbaceous vegetation and shrubs (e. g.: *Hypsiboas pulchellus*, *Scinax squalirostris*). During the off-season, the fields retain water in the irrigation and drainage canals, and there are ephemeral pools in the rice plots. The combination of reduced superficial water depth and the lack of vegetation cover during the off-season could turn the rice fields into a habitat that is unfavorable to these species. Similar patterns have been seen in anuran assemblages in agroecosystems in central Brazil (Piatti et al. 2010). Comparisons between terrestrial and perches species guilds show distinct patterns of changes throughout the rice cultivation cycle. These differences could be attributed to habitat preferences and differences in the dispersal ability of the species. In the study area, all of the terrestrial species are frequently associated with grassland and temporary ponds. Since the water used for crop irrigation comes from streams or permanent ponds, predatory fish such as wolf fish (*Hoplias* spp.), catfish (*Rhamdia* spp.) and thin dogfish (*Oligosarchus* spp.) are frequently found in the plots and irrigation canals. In addition to the predation effect on tadpoles and adults, the presence of fish influences the selection of breeding sites (or habitats) by many amphibians (Both et al. 2009; Resetaris 2005; Werner et al. 2007). In addition to this fact, the rice growing period coincides with the reproductive period of most species. In the rice fields, the behavior of anurans avoiding sites with fish would explain the differences in abundance between the initial crop stage (shallow water level) and the final stage (deep water level). Thus, the greater water depth and homogeneous vegetation during the final cultivation period could reduce the availability of shelter or reproductive sites.

Another factor that could influence the distribution of the terrestrial species is the size of the irrigation canals between the rice fields and the non-cultivated areas. Irrigation canals facilitate the movement of anurans and could serve as breeding sites (Herzon and Helenius 2008; Mazerolle 2004). However, the quality of the canal will determine which species will use them, because a successful use will depend on the characteristics and requirements of the species. The connectivity between terrestrial and aquatic habitats is a key factor for the persistence of anuran populations (Becker et al. 2007; Cushman 2006). Breeding sites that are not connected with terrestrial habitats could act as population sinks due to high juvenile mortality (Rothermel 2004). Many studies have indicated that post-metamorphic juvenile dispersal contributes more than adult dispersal to regional persistence (Guerry and Hunter Jr 2002; Semlitsch 2008). In rice fields, large canals could act as barriers to terrestrial

anuran migration (Kato et al. 2010). Terrestrial species do not have adhesive toe discs, which could hinder their ability to climb the walls of deep canals.

The indicator species pointed by our study are predominantly associated with open vegetation (Colombo et al. 2008; Kwet et al. 2010). Various individuals well-distributed among the locations of a specific category are needed to identify an indicator species in an Indval analysis. The association of perches and aquatic species with natural areas only during the off-season period could be attributed to the reduced area of the ponds during the cultivation period. Although irrigation water is not drawn directly from the sampled ponds, water level is highly affected by precipitation fluctuations and groundwater level. Only one indicator species was associated with conventional rice fields. *Leptodactylus gracilis* is a fossorial species who reproduces in foam nests placed in soil cavities that are subsequently flooded. This fact corroborates the idea that only generalist species with high resilience are able to remain in areas converted to rice fields (Doody et al. 2006; Piatti et al. 2010), because species depend upon the physiological tolerance to adapt to rapid changes in the habitat.

Our hypothesis that the species response to organic agriculture would differ among guilds was not supported. Another important result of our study was that the patterns of variation between natural areas and rice fields differ among guilds. The combination of the specific dispersal ability of each group and the presence of barriers to dispersal, such as deep irrigation canals or presence of fish, could be more important to species composition determination than the factor associated with the management of these agricultural areas. Thus, the direct effect of management practices on anurans could be low (but not necessarily absent) and the abundance in areas close to the fields could determine local abundance in crops. This does not exclude the effect of organic management in contributing to abundance in nearby areas. Considering the lack of knowledge about the effects of agricultural practices on anurans, we defend the incorporation of specific traits of each group when formulating more effective conservation strategies.

2.6 REFERENCES

Anand M, Orlóci L (1996) Complexity in plant communities: the notion and quantification. J Theor Biol 179:179–186.

Andersen A, Eltun R (2000) Long-term developments in the carabid and staphylinid (Col ., Carabidae and Staphylinidae) fauna during conversion from conventional to biological farming. J Appl Entomol 124:515–556.

Anderson GKS, Rundlöf M, Smith HG (2010) Time lags in biodiversity response to farming practices. Asp Appl Biol 100:381–384.

Azambuja IHV, Vernetti JFJ, Magalhães JAM (2004) Aspectos socioeconômicos da produção do arroz. In: Gomes AS, Magalhães JA. (eds) Arroz irrigado no Sul do Brasil. Embrapa Informação Tecnológica, Brasília, pp 23–44

Bambaradeniya CNB, Edirisinghe JP, Silva DN, et al. (2004) Biodiversity associated with an irrigated rice agro-ecosystem in Sri Lanka. Biodivers Conserv 13:1715-1753.

Becker CG, Fonseca CR, Haddad CFB, et al. (2007) Habitat split and the global decline of amphibians. Science 318:1775–1777. doi: 10.1126/science.1149374

Beebee TJC, Griffiths R a. (2005) The amphibian decline crisis: a watershed for conservation biology? Biol Conserv 125:271–285. doi: 10.1016/j.biocon.2005.04.009

Beecher NA., Johnson RJ, Brandle JR, et al. (2002) Agroecology of birds in organic and nonorganic farmland. Conserv Biol 16:1620–1631. doi: 10.1046/j.1523-1739.2002.01228.x

Benton TG, Vickery JA, Wilson JD (2003) Farmland biodiversity: is habitat heterogeneity the key? Trends Ecol Evol 18:182–188.

Blondel J (2003) Guilds or functional groups: does it matter? Oikos 100:223–231.

Both C, Solé M, Santos TG, Cechin SZ (2009) The role of spatial and temporal descriptors for neotropical tadpole communities in southern Brazil. Hydrobiologia 624:125–138. doi: 10.1007/s10750-008-9685-5

Colombo P, Kindel A, Vinciprova G, Krause L (2008) Composição e ameaças à conservação dos anfíbios anuros do Parque Estadual de Itapeva , município de Torres , Rio Grande do Sul , Brasil. Biota Neotropica 8:229–240.

Cosentino BJ, Schooley RL, Phillips C a. (2010) Connectivity of agroecosystems: dispersal costs can vary among crops. Landsc Ecol 26:371–379. doi: 10.1007/s10980-010-9563-1

Crump ML, Scott NJ (1994) Standard techniques for inventoring and monitoring: visual encounters surveys. In: Heyer WR, Donelly MA, McDiarmid RW, et al. (eds) Measuring and monitoring biological diversity. Standard methods for amphibians. Smithsonian Institution Press, Washington, USA, pp 84–92

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biol Conserv 128:231–240. doi: 10.1016/j.biocon.2005.09.031

Devine GJ, Furlong MJ (2007) Insecticide use: contexts and ecological consequences. Agric Human Values 24:281–306.

Dixo M, Metzger JP (2010) The matrix-tolerance hypothesis: an empirical test with frogs in the Atlantic Forest. Biodivers Conserv 19:3059–3071. doi: 10.1007/s10531-010-9878-x

Donald PF (2004) Biodiversity impacts of some agricultural. Conserv Biol 18:17–37.

Doody JS, Osborne W, Bourne D, et al. (2006) Vertebrate biodiversity on Australian rice farms. Rural Industries Research and Development Corporation, Kingston ACT

Duarte LDS, Machado RE, Hartz SM, Pillar VD (2006) What saplings can tell us about forest expansion over natural grasslands. J Veg Sci 17:799-808. doi: 10.1658/1100-9233(2006)17[799:WSCTUA]2.0.CO;2

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monogr 67:345–366.

Duré MI, Kehr AI, Schaefer EF, Marangoni F (2008) Diversity of amphibians in rice fields from northeastern Argentina. Interciencia 33:523–527.

Ernst R, Rödel MO (2008) Patterns of community composition in two tropical tree frog assemblages: separating spatial structure and environmental effects in disturbed and undisturbed forests. J Trop Ecol 24:111–120. doi: 10.1017/S0266467407004737

FAO Stat. (2010) FAO statistical databases. http://faostat.fao.org. Accessed 8 Aug 2012

Ficetola GF, De Bernardi F (2004) Amphibians in a human-dominated landscape: the community structure is related to habitat features and isolation. Biol Conserv 119:219–230. doi: 10.1016/j.biocon.2003.11.004

Fujioka M, Lane SJ (1997) The impact of changing irrigation practices in rice fields on frog populations of the Kanto Plain, central Japan. Ecol Res 12:101–108. doi: 10.1007/BF02523615

Fuller RJ, Norton LR, Feber RE, et al. (2005) Benefits of organic farming to biodiversity vary among taxa. Biol lett 1:431–434. doi: 10.1098/rsbl.2005.0357

Gallant AL, Klaver RW, Casper GS, Lanoo MJ (2007) Global rates of habitat loss and implications for amphibian conservation. Copeia 207:967–979.

Gray MJ, Smith LM, Brenes R (2004) Effects of agricultural cultivation on demographics of southern high plains amphibians. Conserv Biol 18:1368–1377. doi: 10.1111/j.1523-1739.2004.00089.x

Guerry AD, Hunter Jr ML (2002) Amphibian distributions in a landscape of forests and agriculture : an examination of landscape composition and configuration. Conserv Biol 16:745–754.

Hazell D (2003) Frog ecology in modified Australian landscapes: a review. Wildl Res 30:193–205.

Herzon I, Helenius J (2008) Agricultural drainage ditches, their biological importance and functioning. Biol Conserv 141:1171–1183. doi: 10.1016/j.biocon.2008.03.005

Hole DG, Perkins a. J, Wilson JD, et al. (2005) Does organic farming benefit biodiversity? Biol Conserv 122:113–130. doi: 10.1016/j.biocon.2004.07.018

Kato N, Yoshio M, Kobayashi R, Miyashita T (2010) Differential responses of two anuran species breeding in rice fields to landscape composition and spatial scale. Wetlands 30:1171–1179. doi: 10.1007/s13157-010-0103-1

Knutson MG, Richardson WB, Reineke DM, et al. (2004) Agricultural ponds support amphibian populations. Ecol Appl 14:669–684.

Kwet A, Lingnau R, Di-Bernardo M (2010) Anfíbios da serra gaúcha Pró mata. Edipucrs, Porto Alegre

Machado IF, Maltchik L (2010) Can management practices in rice fields contribute to amphibian conservation in southern Brazilian wetlands? Aquat Conserv 46:39–46. doi: 10.1002/aqc.1070

Mann RM, Hyne R V, Choung CB, Wilson SP (2009) Amphibians and agricultural chemicals: review of the risks in a complex environment. Environ pollut 157:2903–2927. doi: 10.1016/j.envpol.2009.05.015

Marsh DM, Thakur K a., Bulka KC, Clarke LB (2004) Dispersal and colonization through open fields by a terrestrial, woodland salamander. Ecology 85:3396–3405. doi: 10.1890/03-0713

Mazerolle MJ (2004) Drainage ditches facilitate frog movements in a hostile landscape. Landsc Ecol 20:579-590.

Oksanen J, Blanchet FG, Kindt R, et al. (2012) vegan: Community Ecology Package. R package version 2. 0. 3. http:// CRAN.R-project.org/package=vegan

Peltzer PM, Lajmanovich RC, Sánchez-Hernandez JC, et al. (2008) Effects of agricultural pond eutrophication on survival and health status of *Scinax nasicus* tadpoles. Ecotoxicol Environ Saf 70:185–197. doi: 10.1016/j.ecoenv.2007.06.005

Piatti L, Souza FL, Filho PL (2010) Anuran assemblage in a rice field agroecosystem in the Pantanal of central Brazil. J Nat Hist 44:1215–1224. doi: 10.1080/00222930903499804

Piha H (2006) Impacts of agriculture on amphibians at multiple scales. Dissertation, University of Helsinki.

R Development Core Team (2012) R: A language and environment for statistical computing. R foundation fo statistical computing, Viena, Austria. http:// www.R-project.org

Resetaris WJ (2005) Habitat selection behaviour links local and regional scales in aquatic systems. Ecol Lett 8:480–486.

Roberts DW (2012) labdsv: ordination and multivariate analysis for ecology. R package version 1. 5-0. http://cran.r-project.org/package=labdsv

Rossato MS (2011) Os climas do Rio Grande do Sul: variabilidade, tendência e tipologia. Tese de doutorado. Universidade Federal do Rio Grande do Sul

Rothermel BB (2004) Migratory success of juveniles: a potential constraint on connectivity for pond-breeding amphibians. Ecol Appl 14:1535–1546.

Rundlo M, Jonason D, Andersson GKS, et al. (2011) Assessing the effect of the time since transition to organic farming on plants and butterflies. J Appl Ecol 48:543–550. doi: 10.1111/j.1365-2664.2011.01989.x

Segalla MV, Caramaschi U, Cruz CAG, et al. (2012) Brazilian amphibians – List of species. www.sbherpetologia.org.br. Accessed 8 Aug 2012

Semlitsch RD (2008) Differentiating migration and dispersal processes for pond-breeding amphibians. J Wildl Manage 72:260–267. doi: 10.2193/2007-082

Vallan D (2000) Influence of forest fragmentation on amphibian diversity in the nature reserve of Ambohitantely , highland Madagascar. Biol Conserv 96:31–43.

Vasconcelos D, Calhoun AJK (2004) Movement patterns of adult and juvenile *Rana sylvatica* (LeConte) and *Ambystoma maculatum* (Shaw) in three restored seasonal pools in Maine. J Herpetol 38:551–561.

Weibull AC, Bengtsson J, Nohlgren E (2000) Diversity of butterflies in the agricultural landscape: the role of farming system and landscape heterogeneity. Ecography 23:743–750. doi: 10.1111/j.1600-0587.2000.tb00317.x

Werner EE, Skelly DK, Relyea R a., Yurewicz KL (2007) Amphibian species richness across environmental gradients. Oikos 116:1697–1712. doi: 10.1111/j.2007.0030-1299.15935.x

Wilson AL, Watts RJ, Stevens MM (2007) Effects of different management regimes on aquatic macroinvertebrate diversity in Australian rice fields. Ecol Res 23:565–572. doi: 10.1007/s11284-007-0410-z

Zahr JH (1999) Biostatistical analysis. Prentice Hall, New Jersey

2.7 FIGURES
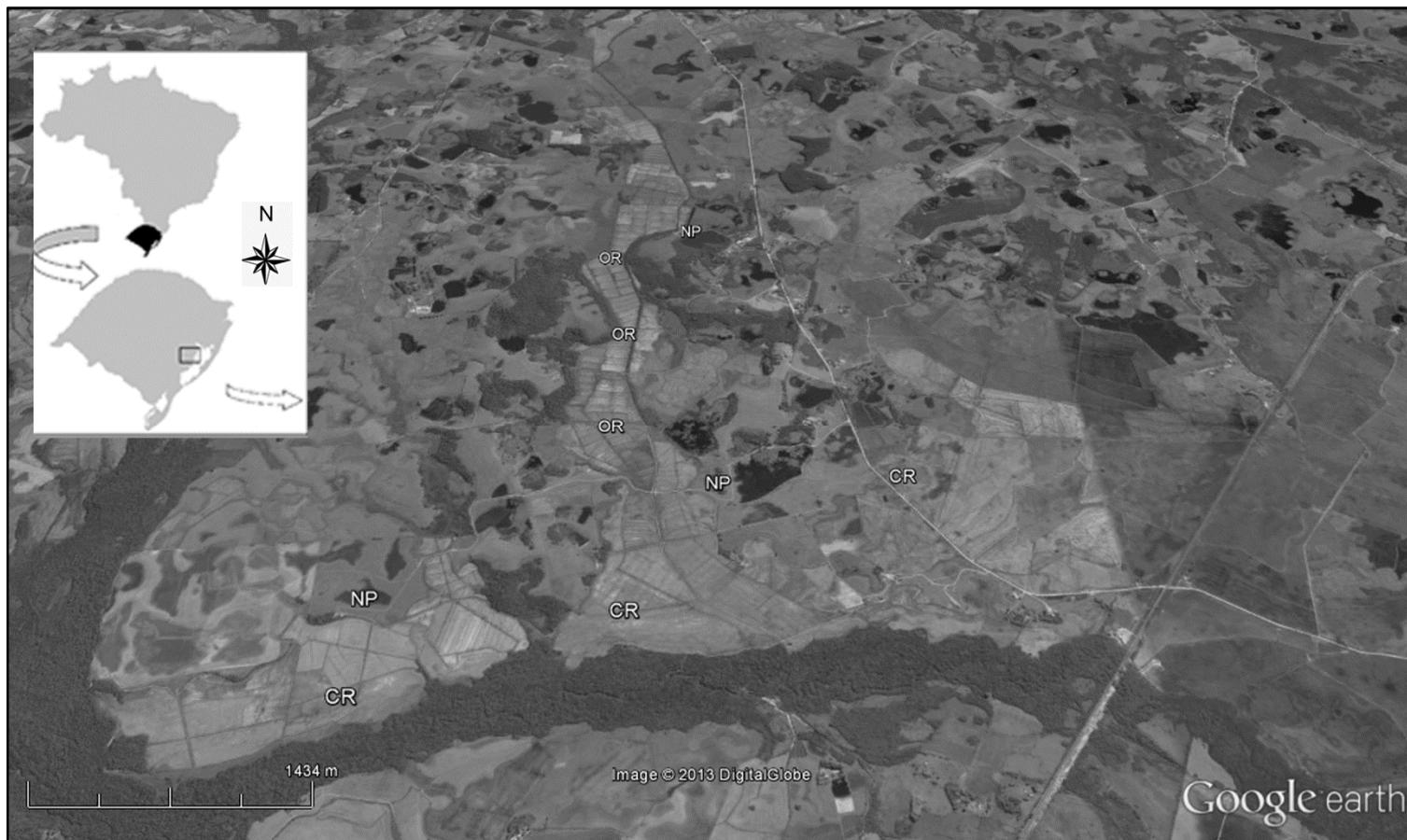
Fig. 1 Map of study area in the coastal plain of Rio Grande do Sul, Brazil. NP: natural ponds, OR: organic rice fields, CR: conventional rice fields
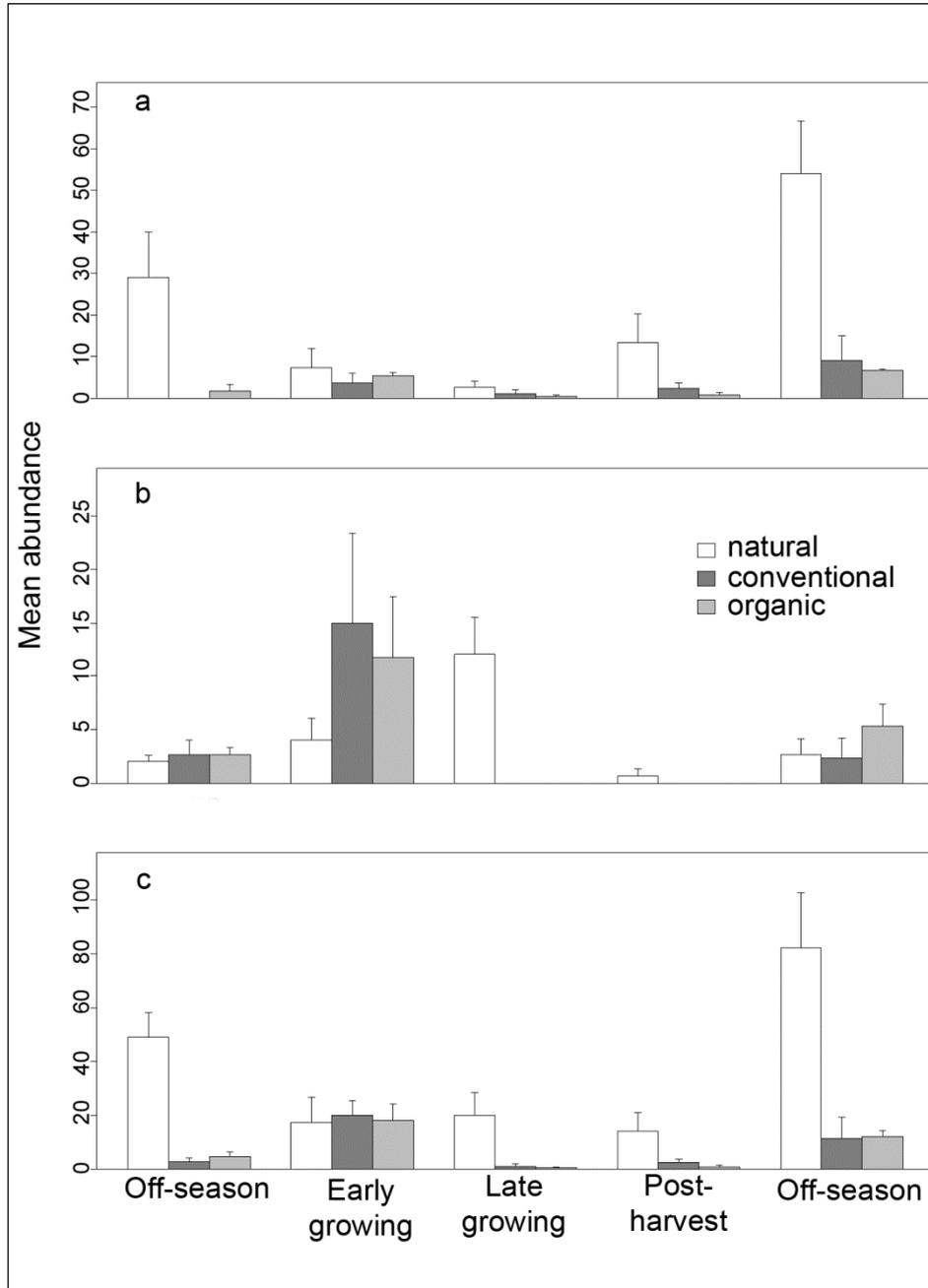
Fig. 2 Mean anuran abundance in a rice paddy area in Sentinela do Sul, RS, Brazil, across the rice cultivation cycle (August/2011 to August/2012). a: perches species, b: terrestrial species, c: whole assemblage
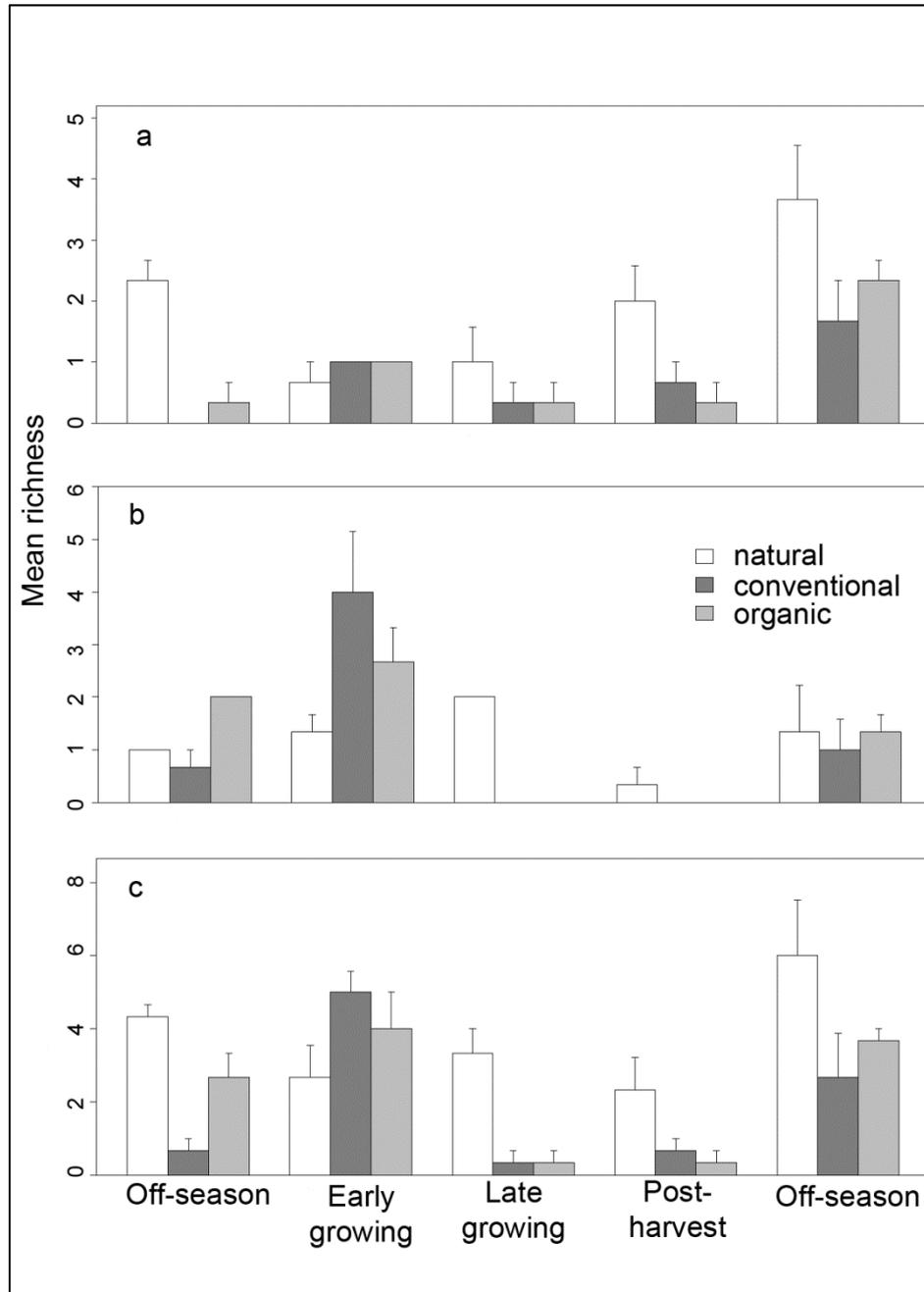
Fig. 3 Mean anuran richness in a rice paddy area in Sentinela do Sul, RS, Brazil, across the rice cultivation cycle (August/2011 to August/2012). a: perches species, b: terrestrial species, c: whole assemblage
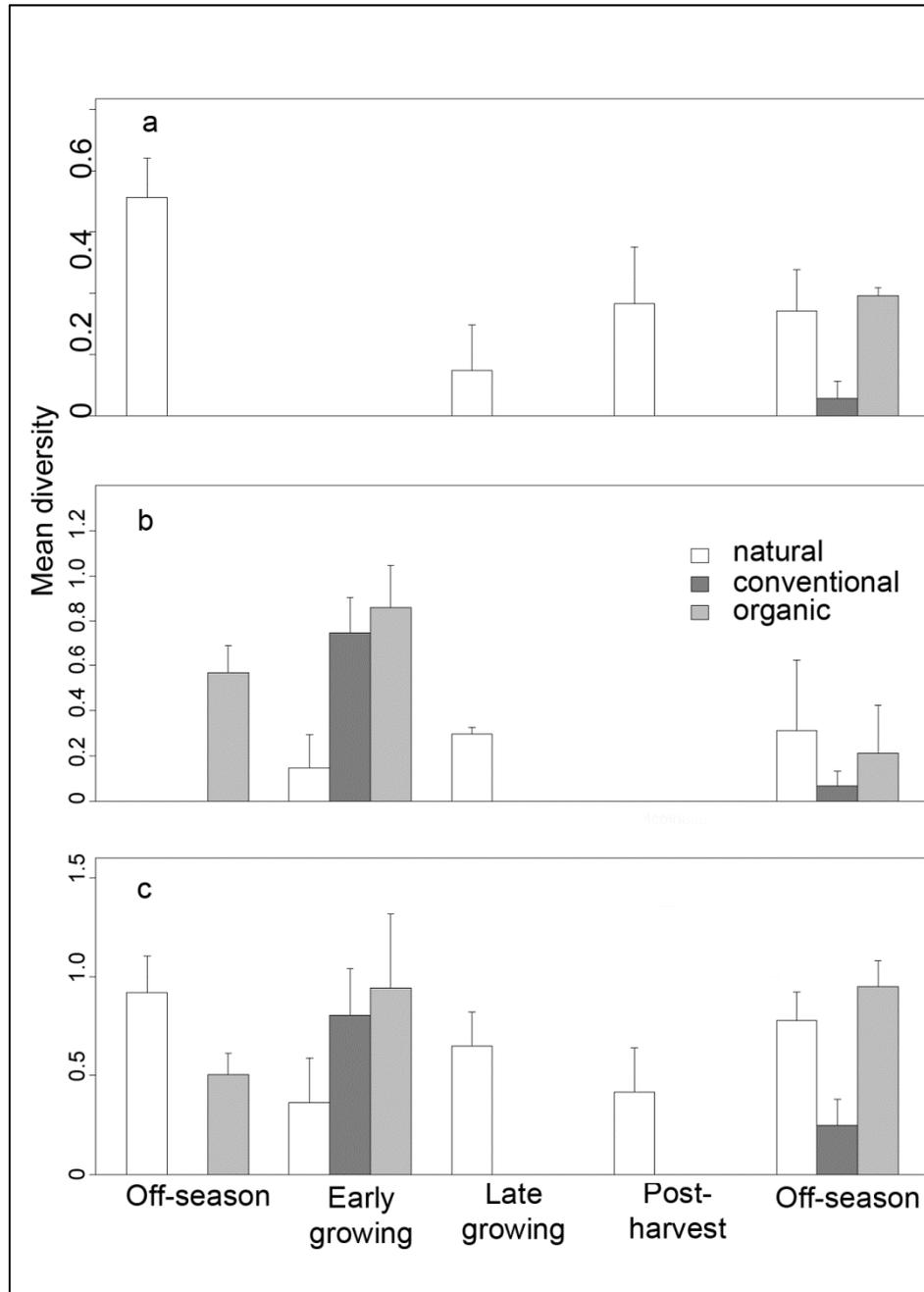
Fig. 4 Mean anuran diversity in a rice paddy area in Sentinela do Sul, RS, Brazil, across the rice cultivation cycle (August/2011 to August/2012). a: perches species, b: terrestrial species, c: whole assemblage
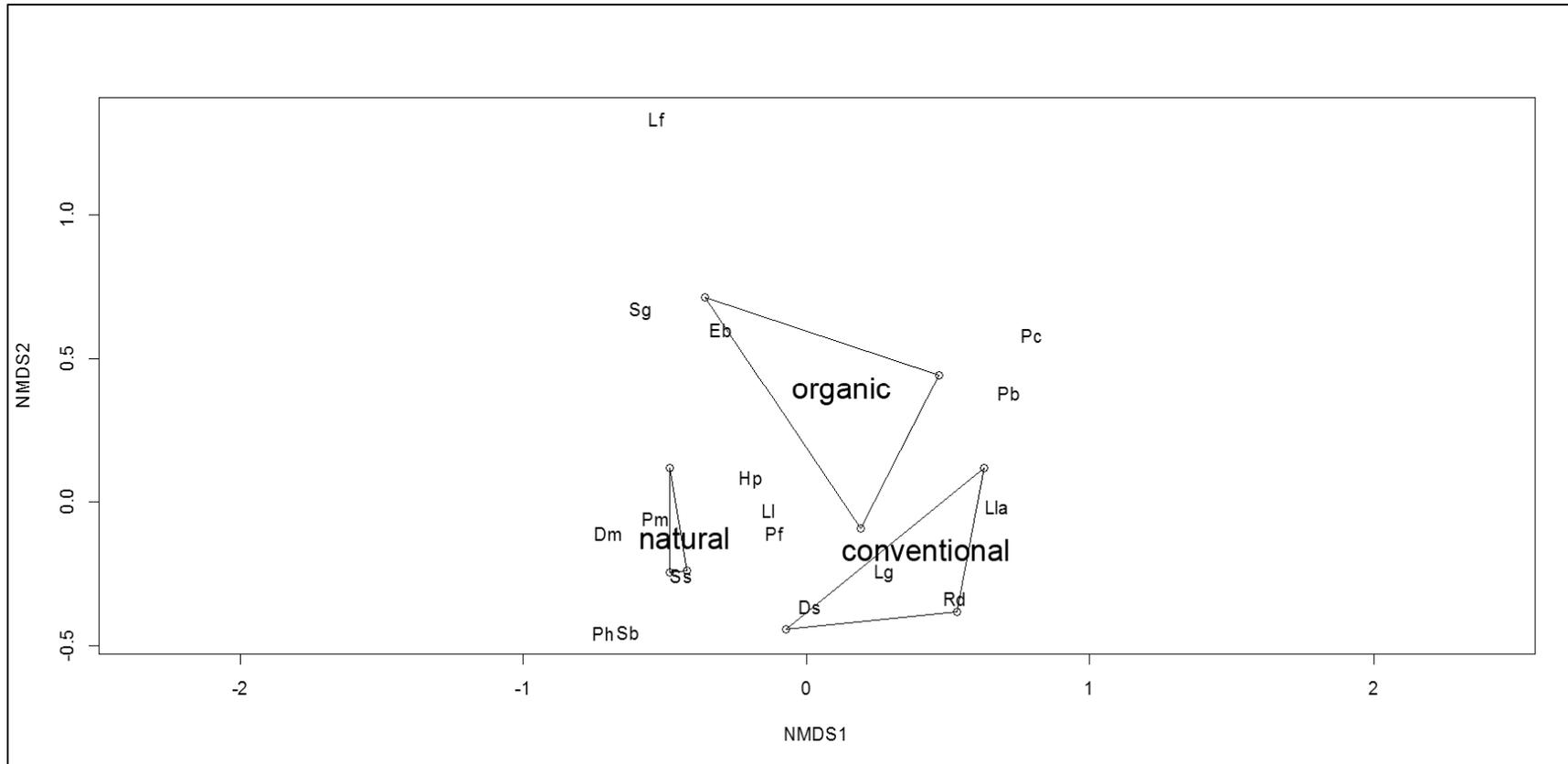
Fig. 5 Multidimensional scaling ordination for an assemblage of anurans in a rice paddy area with different types of management (stress = 0.118),

in Sentinela do Sul, RS, Brazil, from August/2011 to August/2012. Rd= *Rhinella dorbignyi*, Dm = *Dendropsophus minutus*, Ds = *Dendropsophus*

sanborni, Hp= *Hypsiboas pulchellus*, Pm= *Pseudis minuta*, Sb= *Scinax berthae*, Sg= *Scinax granulatus*, Ss= *Scinax squalirostris*, Pb=

Physalaemus biligonigerus, Pc=*Physalaemus cuvieri*, Ph= *Physalaemus henselii*, Pf= *Pseudopaludicola falcipes*, Lf= *Leptodactylus fuscus*, Lg=

Leptodactylus gracilis, Ll= *Leptodactylus latrans*, Lla= *Leptodactylus latinasus*, Eb= *Elachistocleis bicolor*

2.8 TABLES

Table 1. Anuran abundances registered in a rice paddy area in Sentinela do Sul, Rio Grande do Sul, Brazil, from August 2011 to August 2012. A: aquatic, T:

terrestrial, Pe: perches

Species	Guild	Conventional cultivation	Organic cultivation	Natural ponds
Bufonidae				
Rhinella dorbignyi (Duméril & Bibron, 1841)	T	04		02
Hylidae				
Dendropsophus sanborni (Schmidt, 1944)	Pe	41	15	112
Dendropsophus minutus (Peters, 1872)	Pe			09
Hypsiboas pulchellus (Duméril & Bibron, 1841)	Pe	05	22	112
Pseudis minuta Günther, 1858	A		04	165
Scinax berthae (Barrio, 1862)	Pe			16
Scinax granulatus (Peters, 1871)	Pe		04	02
Scinax squalirostris (A. Lutz, 1925)	Pe	02	03	68
Leiuperidae				
Physalaemus biligonigerus (Cope, 1861 "1860")	T	13	08	
Physalaemus cuvieri Fitzinger, 1826	T	03	04	
Physalaemus henselii (Peters,1872)	T			02
Pseudopaludicola falcipes (Hensel, 1867)	T	15	11	34
Leptodactylidae				
Leptodactylus fuscus (Schneider, 1799)	T		01	
Leptodactylus gracilis (Duméril & Bibron, 1841)	T	10	07	02
Leptodactylus latinasus Jiménez de la Espada 1875	T	12	15	
Leptodactylus latrans (Steffen, 1815)	T	01	08	19
Microhylidae				
Elachistocleis bicolor (Valenciennes in Guérin-Menéville,1838)	T	02	05	05

Table 2. Results of ANOVA comparisons between rice fields (conventional and organic) and natural ponds across rice cultivation cycle. * Significant pairwise comparisons (p<0.05). C: conventional cultivation, O: organic cultivation, N: natural ponds, o: off-season, e: early growing, l: late growing

Comparison	Index	Management			Management x crop cycle		
		$F_{(2,30)}$	p	Tukey*	$F_{(8,30)}$	p	Tukey*
Whole assemblage	Richness	11.58	<0.001	C-N,O-N	3.41	0.007	Cl-Nl, Ol-Nl, Co-No
	Abundance	32.09	<0.001	C-N,O-N	3.53	0.005	Co-No, Oo-No
	Diversity	7.74	0.002	C-N,C-O	3.63	0.004	Co-No
Perches	Richness	8.08	0.002	C-N,O-N	2.13	0.06	
	Abundance	20.40	<0.001	C-N,O-N	2.88	0.02	Co-No, Oo-No
	Diversity	19.91	<0.001	C-N,O-N	5.05	<0.001	Co-No, Oo-No
Terrestrial	Richness	1.73	0.194		4.39	0.001	Cl-Nl, Ol-Nl
	Abundance	0.92	0.409		3.77	0.004	Cl-Nl, Ol-Nl
	Diversity	2.99	0.06		3.85	0.003	Oe-Ne

Table 3. PERMANOVA comparing anuran assemblages across natural ponds, conventional rice fields, and organic rice fields and across crop cycle

Comparison	R^2	Df	F	p
Global comparison across all regimes	0.533	2	3.43	**0.034**
Off-season	0.644	2	5.42	**0.005**
Growing	0.337	2	1.53	0.185
Post-harvest	0.267	2	1.09	0.422

Table 4. Indicator species with respective value (Indval), probability for species to be listed as indicator (p), cultivation period and sites where the species showed maximum indicator value

Species	Indval	p	Period	Site
Dendropsophus sanborni	0.518	0.04	Off-season	Natural ponds
Hypsiboas pulchellus	0.633	0.004	Off-season	Natural ponds
Pseudis minuta	0.775	0.002	Off-season	Natural ponds
Scinax squalirostris	0.740	0.005	Off-season	Natural ponds
Pseudopaludicola falcipes	0.45	0.013	Growing	Natural ponds
Leptodactylus gracilis	0.526	0.03	Growing	Conventional rice
Leptodactylus latrans	0.500	0.03	Growing	Natural ponds

3 CAPÍTULO 2

Este capítulo foi submetido na forma de artigo científico ao periódico *Acta Oecologica*.

Amphibians in an agricultural area: the community structure is related to crop age

Leonardo Felipe Bairos Moreira[1,*] and Leonardo Maltchik[1]

[1] Laboratório de Ecologia e Conservação de Ecossistemas Aquáticos, Universidade do Vale do Rio dos Sinos-UNISINOS, Av. Unisinos, 950, 93022-000, São Leopoldo, Brasil

* corresponding author: e-mail: leonardobm@gmail.com ; phone number: 55-51-3591-1100 ; fax number: 55-51-3590-8122

Abstract

Agricultural landscapes provide habitats for many species of amphibians. However, historical factors may affect richness and community structure in these agroecosystems. Here, we examined co-occurrence and nestedness patterns in anuran assemblages in natural areas and rice fields with different land use histories in southern Brazil. The rice fields had different ages (03, 10, and 20 years). We studied whether co-occurrence and nestedness were associated with time since cultivation and whether these patterns were similar across ecomorphological guilds in these areas. We used PERMANOVA to compare the composition in natural areas and rice fields throughout the cultivation cycle. Species co-occurrence patterns were analyzed using the C-score index and two null models. In order to quantify nestedness, we used the metric based on overlap and decreasing fill (NODF). Community composition varied between crop histories, and aquatic and perches species were associated with native areas. Terrestrial species abundance increased throughout the crop age in rice fields. When analyzing all species together, a pattern of segregation was present in all of the fixed-weight models. Perches frogs were always randomly distributed. Segregation and nestedness of terrestrial frogs was always higher when analyses were adjusted for crop age. Our results differed according to the land use history of each area. A local factor associated with crop age could affect distinct groups of species in different ways.

Key words: co-occurrence; nestedness; guilds; rice fields; amphibian conservation; southern Brazil.

3.1 INTRODUCTION

Conversion of natural areas into agricultural areas or permanent pastures has considerably reduced the extent of natural environments in all Brazilian biomes (Ferreira et al., 2012) Consequently, native species abundance has declined and species have become more isolated and at risk of extinction (Green et al. 2005, Dixo et al. 2009). Species in smaller, and more isolated fragments are more likely to decline due to reduced rates of immigration and colonization (Ewers and Didham 2006). There have been conflicting results on the effects of agricultural expansion and intensification on biodiversity (Sparovek et al. 2010). These differences have been attributed to the land use history of the cultivated area, type of crop and development of more sustainable practices.

Species distribution in agricultural areas is influenced by both ecological and historical characteristics of an area (Lunt and Spooner 2005, Piha et al. 2007). So, species richness and community structure are in part related to the historical land use of an area. Community changes resulting from land use are mainly attributed to structural alterations of the soil and vegetation (Dupouey et al. 2002) and the accumulation of agrochemicals that could alter growth rates and parasite infections in resident species (Gray and Smith 2005, Peltzer et al. 2008). Furthermore, many agricultural impacts are related to effects on matrix quality (Donald and Evans 2006), which could affect migration and dispersal processes (Knutson et al. 2004, Ewers and Didham 2006, Donald and Evans 2006). However, agricultural landscapes are characterized by marked changes in the matrix at different temporal scales. Some of these changes are predictable in time (such as harvesting annual crops) or the type of crop (crop rotation). Thus, some agricultural areas are better described as diversified instead of fragmented since the matrix is not necessarily hostile to all species (Ficetola and De Bernardi 2004, Lunt and Spooner 2005, Dixo and Metzger 2010).

Various amphibian species have been able to explore irrigation channels and dams through overland dispersion (Marsh et al. 2004) and may persist as metapopulations in agricultural areas in the absence of less mobile predators (i.e. fish) (Knutson et al. 2004, Herzon and Helenius 2008). Communities may show patterns of co-occurrence and/or nestedness in areas where selective processes such as extinction and colonization are occurring (Ulrich et al. 2009). In a segregated pattern, species tend to co-occur less frequently and many sites result from a unique combination of

species. In a nested pattern, species partially overlap, and in areas with low species diversity, species are subgroups of species that are found in areas with high diversity (Ulrich and Gotelli 2007). These two structural patterns result from biotic (interspecific competition, dispersal ability), abiotic (habitat quality) or historical (differential colonization) factors (Ulrich and Gotelli 2007, Both et al. 2011, Heino 2013). Therefore, patterns of co-occurrence and nestedness could be related to ecological species traits or habitat characteristics, which leads to the formulation of hypotheses about processes of extinction and colonization in the landscape (Ficetola and De Bernardi 2004, Werner et al. 2007, Moreira and Maltchik 2012). Information about these mechanisms are particularly important in areas under agricultural influence where amphibians face a wide array of impacts in both aquatic and terrestrial environments (Cushman 2006, Mann et al. 2009).

Rice fields are important complementary habitats to natural wetlands in many regions of the world and frogs use these agricultural areas to forage and breeding (Duré et al. 2008, Piatti et al. 2010). Nevertheless, the way in which crop age influences anuran community structure has received little attention. Various studies in modified landscapes have shown that the ability of amphibian populations to persist, over time, is related to individual species characteristics (dispersal ability, reproductive modes and habitat preference) (Ficetola and De Bernardi 2004, Cushman 2006, Dixo and Metzger 2010). Thus, frogs that share ecological and/or phylogenetic similarities should respond in a similar manner to habitat modifications. Approaches that focus on particular groups (guilds) could be invaluable if they evaluate the relationship between species composition patterns and the environment (Ernst and Rödel 2008).

In this study, we examined patterns of co-occurrence and nestedness in anuran assemblages in natural areas and rice fields with different cultivation ages. First, we verified differences in the composition of natural wetlands and rice fields and whether these differences were consistent throughout the crop cycle. Then we determined whether the observed patterns of co-occurrence and nestedness were associated with crop age. We also investigated whether the patterns of co-occurrence differed across anuran guilds or stages of the crop cycle. We hypothesized that i) frog assemblages in rice fields will show significant nestedness and this pattern would be guided by crop age (suggesting the predominance of extinction-colonization dynamics); ii) anuran guilds will be distinctly affected by

crop age as a result of their ability to disperse through the agricultural matrix . Thus, terrestrial species could be confined within cultivated areas, aquatic species will disperse only during rice growing period and perches species will not be confined within cultivated areas.

3.2 METHODS

Study area

The state of Rio Grande do Sul produces 67.5% of the rice produced in Brazil, and the coastal plain region is an important area for irrigated rice production in South America (Azambuja et al. 2004). The study occurred between August 2011 and August 2012 in an agricultural area (30.705° to 30.755°S ; 51.630° to 51.700°W) dominated by rice production, in the central-west portion of the coastal plain. The climate is mildly humid subtropical and the average temperature is 18.5°C, with an average of 11°C in winter and 26°C in summer. Annual rainfall ranges from 1500 to 1700 mm/year.

Site selection and data collection

Rice plantations are divided into multiple 1 ha plots that are interconnected by secondary roads and drainage canals. These drainage canals (2-5 m wide and 0.5-1.5 m deep) are filled with water from nearby streams and provide water to the cultivated plots (~ 10 cm water for 130 days) during the rice cultivation cycle. We classified the rice fields according to three crop ages: long-term (areas cultivated for at least 20 years), mid-term (areas cultivated for up to 10 years) and short-term (areas cultivated no more than three years). Three replicas of rice parcels for each cultivation time were sampled within a 10 km radius (Fig. 1). Plots next to other types of vegetation were avoided since we were interested in species associated with rice fields. We sampled three ponds to compare the composition between natural and modified areas. We aimed at including natural areas with the same size and hydroperiod (less than five months) as rice fields. Study sites were at least 600 m from one another to minimize spatial autocorrelation. The spatial independence of the twelve sampling areas was tested using PCNM analysis. As the PCNM had not detected significant spatial structure (P = 0.46), it was not included in the statistical analysis.

We sampled five times during the rice cultivation cycle: two times during the off season (August/2011 and August/2012), two times during the growth period (January/2012 and March/2012) and once during post-harvest (June/2012). We used visual and auditory search techniques to measure anuran richness and abundance. We sampled between 19:00h and 24:00h, and the location sampling order was random. Each sampling consisted of following a 100m transect perpendicular to the area being sampled (rice fields and ponds) for 20 minutes. All individuals that were located visually or acoustically were recorded. Pilot studies showed that 20 minutes was sufficient to sample one rice field plot. The species were classified in guilds according to Vallan (2000): (aquatic) found floating on the water surface; (terrestrial) found on the ground; (perches) found in herbaceous and shrubby habitats.

Statistical analyses

We used non-metric multidimensional scaling (NMDS) to evaluate the differences in anuran composition among natural areas and rice fields. The similarity matrices were calculated using Bray-Curtis distance and scaling focused on two axes. We tested the significance of any composition difference perceived by NMDS with a permutational variance analysis (PERMANOVA). The similarity matrices were calculated with Bray-Curtis distance with 1000 permutations. Differences between cultivation ages were also analyzed separately between the periods of off season, growth and post-harvest. Analyses were carried out on vegan package 2.0.3 for R (Oksanen et al. 2012).

Since the species groups could be subjected to different environmental pressures, we analyzed co-occurrence in two ways: considering all species as equivalent and separating species into guilds. Temporal segregation is a well-documented phenomenon in anurans, so some species could use the same area in different periods and not actually co-occur. Thus, we compiled data regarding the temporal factor jointly: the whole sampling period and each cultivation period separately. We constructed a presence-absence matrix for each class and period with areas in columns and species in rows. We calculated the C-score to measure co-occurrence. A community distributed in patches should yield a higher C-score (hereinafter CS) than expected by chance. Thus, the higher the CS, the

lower the average co-occurrence between pairs of species. Of the metrics available for co-occurrence, CS has a greater statistical power for detecting non-random patterns (Gotelli 2000).

We tested the significance of the CS with two null models. In the first model (fixed-fixed), the column and row totals were fixed. Thus, each randomized area contained the same number of species as the original and each species occurred with the same frequency as the original community (Gotelli and Elllinson 2002). The fixed-fixed model holds the pattern of rich and poor areas in the randomized matrices. This is a traditional null model, although it is highly conservative (Gotelli 2000). In the second null model (fixed-weighted), the columns were adjusted for crop age. If species were associated with different characteristics of the area, the co-occurrence could be less than expected by chance. For example, while one species could prefer dryer locations, another may prefer more humid areas. In this model, we incorporated the importance of the history of an area as an environmental descriptor of community structure. For all of the null models, the random matrices were produced by shuffling the original matrix through repeated random submatrices. In all of the co-occurrence analyses, 5000 random matrices were constructed and the average and standard deviation was calculated for all of the indices. We then evaluated statistical significance by comparing the observed value of the original matrix with the values derived from the random matrices. To facilitate the comparison between different combinations of data, we calculated the standardized effect size (SES) (Gotelli and Mccabe 2002). The interpretations were based on the SES values since CS values are affected by matrix size. The species co-occurrence analyses and associated randomizations were carried out in Ecosim7 (Gotelli and Entsminger 2004).

For nestedness analyses, we built presence and absence matrices where columns and rows were species and areas, respectively. We quantified the nestedness pattern through NODF (Almeida-Neto et al. 2008) as was recommended by Ulrich et al. (2009). NODF is a consistent metric that allows access to nestedness among locations only or among species only. Since our focus was the dissimilarity of the species composition, we only calculated the NODF value between locations. NODF can range from 0 to 100, and higher values indicate more nested communities. Nestedness analyses require that the incidence matrix be ordered according to pre-defined criteria. Thus, we set incidence matrix according two ways: order rows and columns by species frequencies and an historic-

sorted matrix. The different outcomes after sorting can be used to judge whether the pattern is connected to agricultural modifications. Nestedness analyses were also run with either all of the species as equivalents or separating them by guilds. To evaluate the significance of nestedness we used a null model with quasiswap algorithm and 5000 simulations to validate the model significance. The method "quasiswap" maintains the frequency of rows and columns. All of the analyses were carried out on vegan package 2.0.3 for R (Oksanen et al. 2012).

3.3 RESULTS

We documented a total of 16 species of anurans which were distributed among five families (Table 1). Nine of these species were terrestrial. The group of perches frogs was composed of Hylidae species, with the exception of *Pseudis minuta*. Since *P. minuta* was the only predominantly aquatic species in the study area, it was only included in analyses with all species. *Pseudopaludicola falcipes* had a similar abundance in all of the sample areas. *Dendropsophus sanborni*, *Hypsiboas pulchellus* and *P. minuta* were the most abundant species in natural areas. *D. sanborni* was the dominant species in short-term rice fields. Species abundance in mid-term rice fields was relatively homogeneously distributed (Table 1). *Rhinella dorbignyi* was more abundant and frequent in long-term rice fields than in the other areas.

In the NMDS ordination (Fig. 2), rice fields and natural areas formed well-defined groups. Frog communities in short- and mid-term rice fields had similar compositions. Aquatic and perches species were abundant in natural areas. While perches and terrestrial species were associated with short- and mid-term rice fields, only terrestrial species were associated with long-term rice fields. The PERMANOVA of the whole cultivation cycle showed significant differences between natural areas and different cultivation ages (F= 2.907, df= 2, p = 0.015). When separated by cultivation stages, the assemblages only differed during the off season and growth periods (Table 2). During the off season, natural areas formed a distinct group with higher arboreal species abundance than the rice fields (Fig. 3A). During the growth period, the composition of short- and mid-term rice fields was similar to that of natural areas (Fig. 3B). Small perches species such as *D. sanborni* and *Scinax squalirostris* were common in natural areas during the post-harvest period (Fig. 3C).

Only one analysis based on the fixed-fixed algorithm was significant (p< 0.05). The terrestrial anurans were significantly segregated during the rice growth period (Fig. 4A). The set of all species in the community showed a significant pattern of segregation in all of the fixed-weighted analyses (Table 3, Fig. 4B). The co-occurrence of perches species was random in all analyses. The terrestrial species were significantly segregated in all periods except post-harvest (Table 3). The standardized effect size (SES) values were higher in the analyses that included crop age, except for the perches guild. Terrestrial frogs were more segregated than the whole community during the off season period (Fig. 4B). The nestedness models showed differences according to matrix sorting. When the ordination matrices were based on species frequency, none of the matrices showed significant nestedness (Table 4). The ordination matrices based on cultivation age showed significant nestedness for all of the classes except the perches guild.

3.4 DISCUSSION

Anuran assemblages exhibited nonrandom patterns that were contingent on the crop age. This structure was relatively independent of the seasonal availability of water in the rice fields. Although some models showed significant segregation, we observed significant patterns of nestedness depending on the guild under investigation. Segregated distributions are oftentimes attributed to biotic interactions or environmental filters (Ulrich and Gotelli 2007), although other mechanisms could be associated with the nonrandom distribution patterns. Among these mechanisms, environmental heterogeneity and dispersal limitations are particularly important in metacommunities (Richter-Boix et al. 2007, Heino 2013). In agricultural landscapes, local extinctions are common and the biota depends on proximate semi-natural habitats functioning as populations sources (Tscharntke et al. 2005). Although perches frogs never showed any structure in this study, indicating that their communities are random, terrestrial frogs had a segregated structure related to crop age. Our data suggest that a local factor associated with crop age could affect species in different ways.

Perches and aquatic species were more associated with natural areas. Rice fields had a structured pattern with terrestrial species abundance increasing over the cultivation age. Depending on the production method used, rice plantations may create a mosaic of suitable microhabitats for frogs

that are defined by water availability and plant species composition (Donald 2004, Duré et al. 2008). Various studies have shown that organisms that inhabit rice fields are opportunistic or resilient (Doody et al. 2006, Piatti et al. 2010). Terrestrial species may be more frequent in older rice fields because they are resilient to the high level of disturbances in agroecosystems. This idea is supported by the fact that the three species most frequently encountered in the mid and long-term rice fields (*Leptodactylus latinasus*, *Physalaemus biligonigerus*, and *Rhinella dorbignyi*) use burrows in the ground (Maneyro and Carreira 2012). This strategy minimizes desiccation stress caused by higher substrate temperatures that result from the lack of complex vegetation cover.

Our results showed that perches frogs were randomly distributed. This result suggests that the crop age does not affect segregation or aggregation. Many of the species were found in natural areas and rice fields but were less abundant in cultivated areas. This difference could be due to stochastic extinction events associated with rice cultivation and posterior colonization of the farms. Although they are capable of occupying different microhabitats in the rice fields, these species may have a restricted spatial distribution within them. Our results show that species segregation of terrestrial anurans was clearly stronger when the analyses were adjusted for crop age. These patterns were consistent throughout the crop cycle, which indicates spatial, and not temporal, segregation. Species that are tolerant to rice fields may also have a limited distribution in response to factors related to crop age. Amphibians occur less frequently in areas that have been farmed for longer periods of time (Piha et al., 2007). Thus, cultivation time of an area would reflect the matrix quality. In addition to more modifications in areas around plantations, a longer land-use historic implies a longer history of agrochemicals use, which may have lethal and sub-lethal effects on amphibian populations (Mann et al. 2009).

The significant nestedness associated with crop age suggests a significant limiting factor of frog distribution. Patterns of nestedness are expected in fragmented habitats because habitat patches have different sizes and degrees of isolation (Ulrich et al. 2009). Although rice fields are highly connected and dynamic systems, extinction could be a mechanism that causes nestedness in frog assemblages in agroecosystems, such as other temporary ecosystems (Ficetola and De Bernardi 2004, Tockner et al. 2006, Moreira and Maltchik 2012). Irrigated rice fields, being temporary aquatic

habitats with a predictable dry phase, can be highly variable at vegetation cover and structure, predation risk associated, or resource availability (Bambaradeniya et al. 2004, Machado and Maltchik 2010, Piatti et al. 2010). Therefore, crop age could be seen as a historic of disturbances, which selective advantage some taxa and disadvantage others. In our study, anuran guilds were affected differently, and perches frogs did not have significant nestedness independent of matrix sorting. Upon comparison of only the significant results, the whole community was more nested than the terrestrial guild alone. This may be due to the accumulation of species with characteristics that enable them to respond positively to disturbances caused by agricultural intensification.

Theoretical models that recognize matrix heterogeneity, such as species-sorting and mass-effect (Leibold et al. 2004), are useful for explaining patterns in agroecosystems. Species-sorting and mass-effect models differ in terms of the importance of dispersal rates (emigration and immigration). Cultivated fields are frequently compared with islands with low species diversity that depend on colonization from the regional species pool (Roschewitz et al. 2005). Thus, anuran dynamics in rice fields would be more consistent with the scenario predicted by mass effect models (Leibold et al. 2004). Although we did not measure the degree of isolation among areas, amphibian dispersal rates are more strongly affected by terrestrial preferences of the adults and juveniles than distance or connectivity among areas (Rothermel 2004). Furthermore, studies show that post-metamorphic juvenile dispersal contributes more than adult dispersal to regional persistence (Guerry and Hunter Jr 2002, Semlitsch 2008). Large irrigation canals in rice fields may act as barriers to terrestrial anuran migration (Kato et al. 2010). Terrestrial species do not have feet with digital disks, making it difficult for they climb up the wall of deep canals once they dropped off.

Frogs may benefit from the presence of irrigation canals since these canals not only serve as breeding sites but also ensure moist conditions to facilitate movements of juveniles and adults (Mazerolle 2004, Duré et al. 2008). Yet, the quality of a canal depends on the characteristics required by the species that will use it. Thus, a poor quality canal could act as a sink to amphibian populations and may not enable the exchange of individuals between fragments (Herzon and Helenius 2008). Another factor that could limit frog movements is the presence of fish in irrigation canals. Previous studies in amphibian communities suggest that fish predation influences amphibian richness and

distribution (Baber et al. 2004, Werner et al. 2007). Amphibians that reproduce in areas inhabited by fish have tadpoles that are either toxic (i.e. bufonids) or have cryptic behavior (i.e. leiuperids). However the effectiveness of these defense strategies is context dependent, varying with predation type and co-occurring species (Nomura et al. 2011). Predatory fish such as wolf fish (*Hoplias* spp.), catfish (*Rhamdia* spp.), and thin dogfish (*Oligosarchus* spp.) were frequently found in the rice fields and irrigation canals of our study area. We would expect that the patterns in rice fields arise due to the accumulation of species with traits that can achieve positive fitness at different points along predation pressure.

To conclude, land use histories was related to species nestedness and co-occurrence in anuran assemblages in rice fields, and there was a higher occurrence of terrestrial species in long-term fields. Permeability of the agricultural matrix and the potential consequences on species dispersal seem to be the principal factors limiting species occurrence in these agroecosystems. However, this idea is still speculative since rates of dispersal and colonization of each species need to be accurately estimated. Furthermore, metacommunities with the same structure could exhibit different dynamics in different landscapes (Richter-Boix et al. 2007). Regardless of the actual mechanism, a distribution pattern related to land use history was revealed and deserves attention, especially since the patterns differ between groups with distinct life strategies. Therefore, our results contribute to species conservation in agricultural areas.

3.5 ACKNOWLEDGEMENTS

3.6 REFERENCES

Almeida-Neto, M. et al. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. - Oikos 117: 1227–1239.

Azambuja, I. H. V. et al. 2004. Aspectos socioeconômicos da produção do arroz. - In: Gomes, A. S. and Magalhães, J. A. . (eds), Arroz irrigado no sul do Brasil. Embrapa Informação Tecnológica, pp. 23–44.

Baber, M. J. et al. 2004. The relationship between wetland hydroperiod and nestedness patterns in assemblages of larval amphibians and predatory macroinvertebrates. - Oikos 107: 16–27.

Bambaradeniya, C. N. B. et al. 2004. Biodiversity associated with an irrigated rice agro-ecosystem in Sri Lanka. - Biodivers. Conserv. 13: 1715–1753.

Both, C. et al. 2011. Tadpole co-occurrence in ponds: when do guilds and time matter? - Acta Oecol.37: 140–145.

Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. - Biol. Conserv. 128: 231–240.

Dixo, M. and Metzger, J. P. 2010. The matrix-tolerance hypothesis: an empirical test with frogs in the Atlantic Forest. - Biodivers. Conserv. 19: 3059–3071.

Dixo, M. et al. 2009. Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. - Biol. Conserv. 142: 1560–1569.

Donald, P. F. 2004. Biodiversity impacts of some agricultural. - Conserv. Biol. 18: 17–37.

Donald, P. F. and Evans, A. D. 2006. Habitat connectivity and matrix restoration: the wider implications of agri-environment schemes. - J. Appl. Ecol. 43: 209–218.

Doody, J. S. et al. 2006. Vertebrate biodiversity on Australian rice farms. - Rural Industries Research and Development Corporation.

Dupouey, J. L. et al. 2002. Irreversible impact of past land use on forest soils and biodiversity. - Ecology 83: 2978–2984.

Duré, M. I. et al. 2008. Diversity of amphibians in rice fields from northeastern Argentina. - Interciencia 33: 523–527.

Ernst, R. and Rödel, M. O. 2008. Patterns of community composition in two tropical tree frog assemblages: separating spatial structure and environmental effects in disturbed and undisturbed forests. - J. Trop. Ecol. 24: 111–120.

Ewers, R. M. and Didham, R. K. 2006. Confounding factors in the detection of species responses to habitat fragmentation. - Biol. Rev. 81: 117–142.

Ferreira, J., Pardini, R., Metzger, J.P., Fonseca, C.R., Pompeu, P.S., Sparovek, G., Louzada, J., 2012. Towards environmentally sustainable agriculture in Brazil: challenges and opportunities for applied ecological research. J. Appl. Ecol. 49, 535–541.

Ficetola, G. F. and De Bernardi, F. 2004. Amphibians in a human-dominated landscape: the community structure is related to habitat features and isolation. - Biol. Conserv. 119: 219–230.

Gotelli, N. J. 2000. Null model analysis of species co-occurrence patterns. - Ecology 81: 2606–2621.

Gotelli, N. J. and Elllinson, A. M. 2002. Assembly rules for New England ant assemblages. - Oikos 99: 591–599.

Gotelli, N. J. and Mccabe, D. J. 2002. Species co-occurrence: a meta-analysis of J . M . Diamond's assembly rules model. - Ecology 83: 2091–2096.

Gotelli, N. J. and Entsminger, G. L. 2006. EcoSim: null models software for ecology. Version 7. – Acquired Intelligence Inc. & Kesey-Bear, Jericho, VT 05465. http://homepages.together.net/~gentsmin/ecosim.htm.

Gray, M. J. and Smith, L. M. 2005. Influence of land use on postmetamorphic body size of Playa lake amphibians. - J. Wildl. Manage. 69: 515–524.

Green, R. E. et al. 2005. Farming and the fate of wild nature. -Science 307: 550–555.

Guerry, A. D. and Hunter Jr, M. L. 2002. Amphibian distributions in a landscape of forests and agriculture: an examination of landscape composition and configuration. - Conserv. Biol. 16: 745–754.

Heino, J. 2013. Environmental heterogeneity, dispersal mode, and co-occurrence in stream macroinvertebrates. - Ecol. Evol. 3: 344–355.

Herzon, I. and Helenius, J. 2008. Agricultural drainage ditches, their biological importance and functioning. - Biol. Conserv. 141: 1171–1183.

Knutson, M. G. et al. 2004. Agricultural ponds support amphibian populations. - Ecol. Appl. 14: 669–684.

Kato N. et al. 2010 Differential responses of two anuran species breeding in rice fields to landscape composition and spatial scale. - Wetlands 30:1171–1179.

Leibold, M. A. et al. 2004. The metacommunity concept: a framework for multi-scale community ecology. - Ecol. Lett. 7: 601–613.

Lunt, I. D. and Spooner, P. G. 2005. Using historical ecology to understand patterns of biodiversity in fragmented agricultural landscapes. - J. Biogeogr. 32: 1859–1873.

Machado, I. F. and Maltchik, L. 2010. Can management practices in rice fields contribute to amphibian conservation in southern Brazilian wetlands? - Aquat. Conserv. 46: 39–46.

Maneyro, R. and Carreira, S. 2012. Guía de anfíbios del Urugay. - Ediciones de la Fuga.

Mann, R. M. et al. 2009. Amphibians and agricultural chemicals: review of the risks in a complex environment. - Environ. Pollut. 157: 2903–2927.

Marsh, D. M. et al. 2004. Dispersal and colonization through open fields by a terrestrial, woodland salamander. - Ecology 85: 3396–3405.

Mazerolle, M. J. 2004. Drainage ditches facilitate frog movements in a hostile landscape. - Landsc. Ecol. 20: 579–590.

Moreira, L. F. B. and Maltchik, L. 2012. Assessing patterns of nestedness and co-occurrence in coastal pond anuran assemblages. - Amphibia-Reptilia 33: 261–271.

Nomura, F. et al. 2011. Are you experienced? Predator type and predator experience trade-offs in relation to tadpole mortality rates. - J. Zool. 284: 144–150.

Oksanen, J. et al. 2012. Vegan: community ecology package. – R package version 2. 0-3. http://CRAN.R-project.org/package=vegan

Peltzer, P. M. et al. 2008. Effects of agricultural pond eutrophication on survival and health status of *Scinax nasicus* tadpoles. - Ecotoxicol. Environ. Saf. 70: 185–197.

Piatti, L. et al. 2010. Anuran assemblage in a rice field agroecosystem in the Pantanal of central Brazil. - J. Nat. Hist. 44: 1215–1224.

Piha, H. et al. 2007. Amphibian occurrence is influenced by current and historic landscape characteristics. - Ecol. Appl. 17: 2298–2309.

Richter-Boix, A. et al. 2007. Structure and dynamics of an amphibian metacommunity in two regions. - J. Anim. Ecol. 76: 607–18.

Roschewitz, I. et al. 2005. The effects of landscape complexity on arable weed species diversity in organic and conventional farming. - J. Appl. Ecol. 42: 873–882.

Rothermel, B. B. 2004. Migratory success of juveniles: a potential constraint on connectivity for pond-breeding amphibians. - Ecol. Appl. 14: 1535–1546.

Semlitsch, R. D. 2008. Differentiating migration and dispersal processes for pond-breeding amphibians. - J. Wildl. Manage. 72: 260–267.

Sparovek, G. et al. 2010. Brazilian agriculture and environmental legislation: status and future challenges. - Environ. Sci. Technol. 44: 6046–53.

Tockner, K. et al. 2006. Amphibian diversity and nestedness in a dynamic floodplain river (Tagliamento , NE-Italy). - Hydrobiologia 565: 121–133.

Tscharntke, T. et al. 2005. Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management. - Ecol. Lett. 8: 857–874.

Ulrich, W. and Gotelli, N. J. 2007. Disentangling community patterns of nestedness and species co-occurrence. - Oikos 116: 2053–2061.

Ulrich, W. et al. 2009. A consumer's guide to nestedness analysis. - Oikos 118: 3–17.

Vallan, D., 2000. Influence of forest fragmentation on amphibian diversity in the nature reserve of Ambohitantely , highland Madagascar. - Biol Conserv. 96: 31–43.

Werner, E. E. et al. 2007. Amphibian species richness across environmental gradients. - Oikos 116: 1697–1712.

3.7 FIGURES

Figure 1. Map of study area in the coastal plain of Rio Grande do Sul, Brazil. NP: natural ponds, ST: short-term rice, MT: mid-term rice, LT: long-term rice
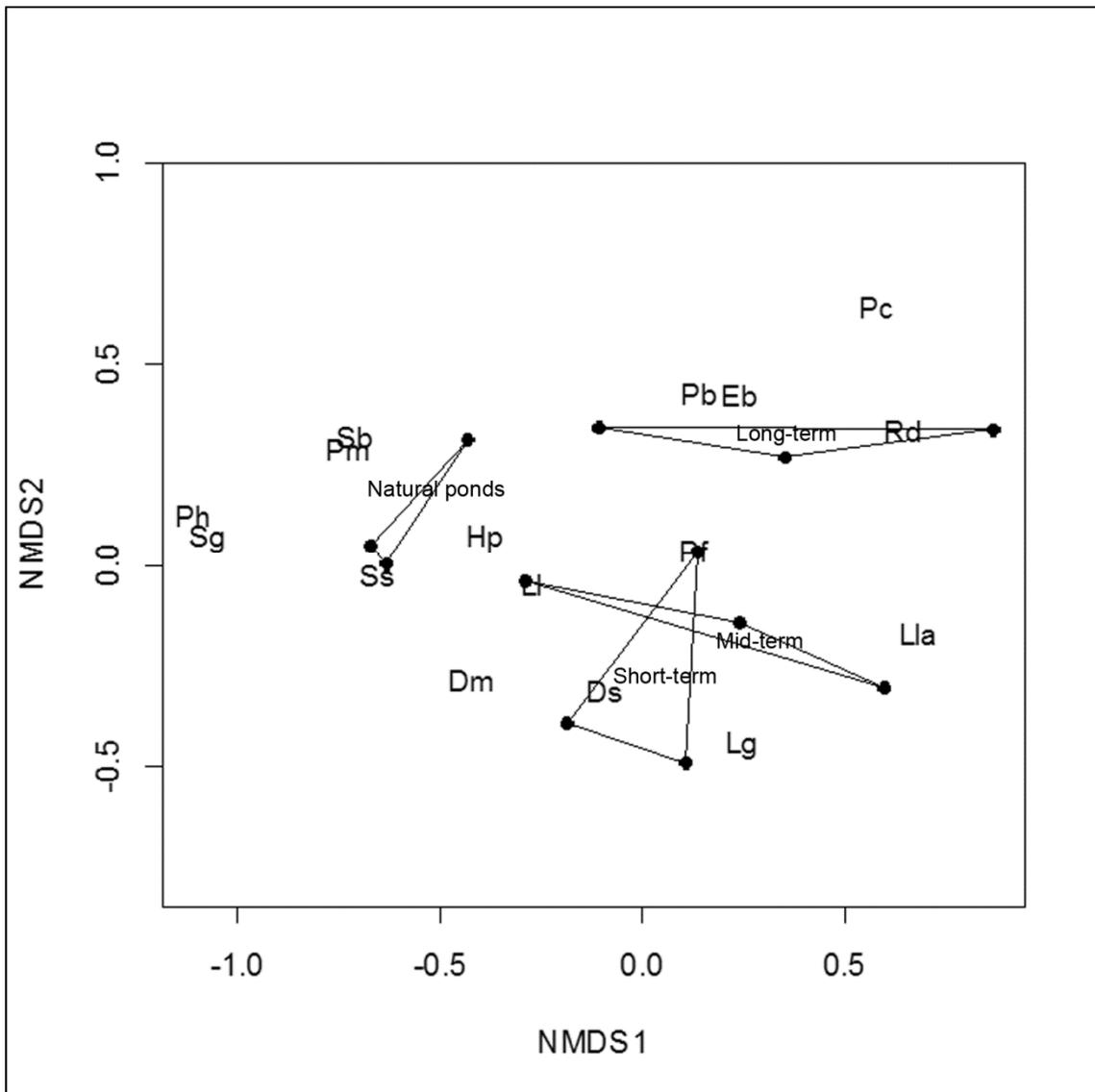
Figure 2. Multidimensional scaling ordination for anuran assemblages in a rice paddy area with different crop ages (stress = 0.125), Rio Grande do Sul, Brazil, from August/2011 to August/2012. Short-term= 03 years, Mid-term=10 years, Long-term: 20 years. Rd= *Rhinella dorbignyi*, Dm= *Dendropsophus minutus*, Ds = *Dendropsophus sanborni*, Hp= *Hypsiboas pulchellus*, Pm= *Pseudis minuta*, Sb= *Scinax berthae*, Sg= *Scinax granulatus*, Ss= *Scinax squalirostris*, Pb= *Physalaemus biligonigerus*, Pc=*Physalaemus cuvieri*, Ph= *Physalaemus henselii*, Pf= *Pseudopaludicola falcipes*, Lg= *Leptodactylus gracilis*, Ll= *Leptodactylus latrans*, Lla= *Leptodactylus latinasus*, Eb= *Elachistocleis bicolor*
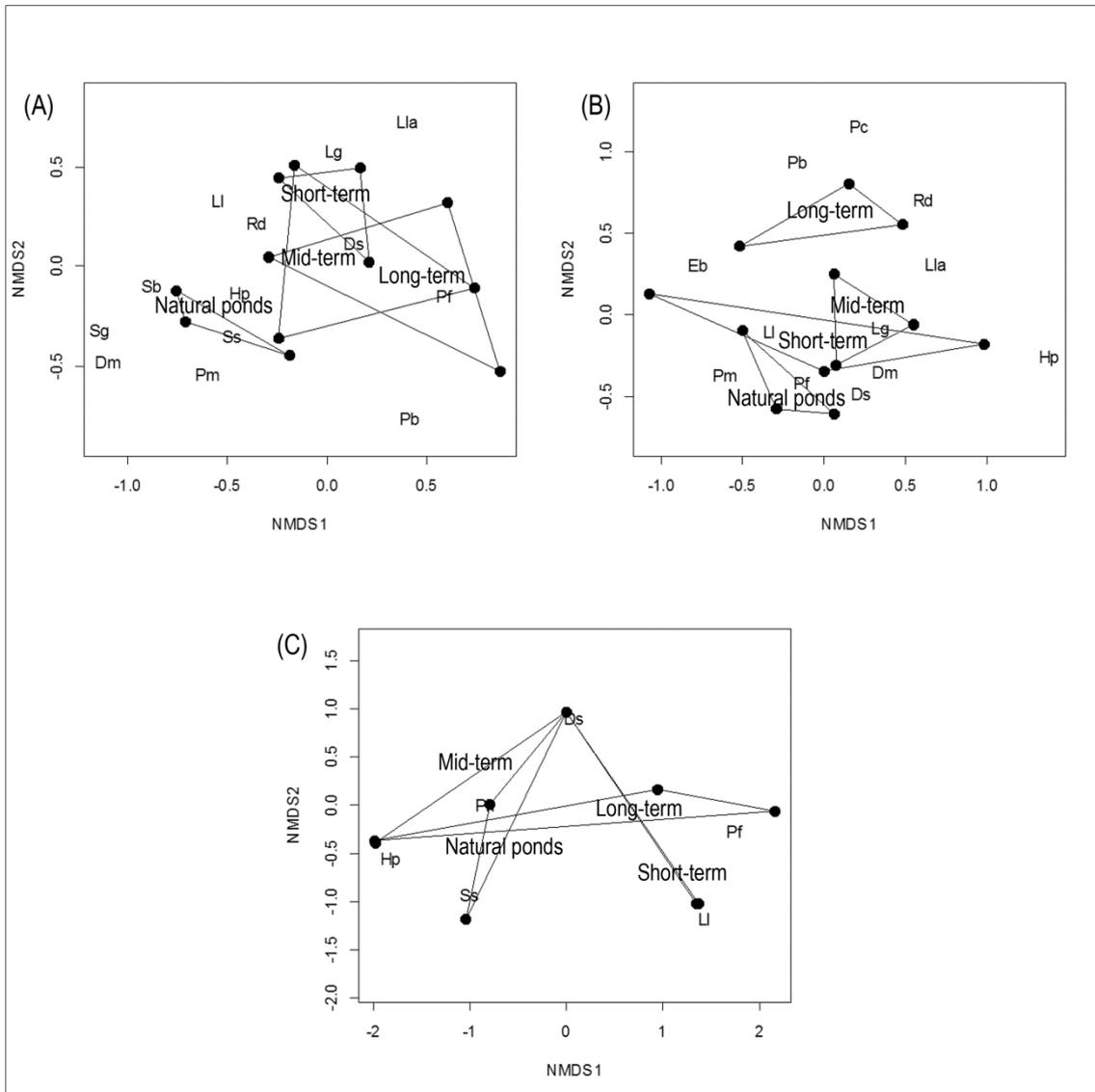
Figure 3. Multidimensional scaling ordination for anuran assemblages across rice cultivation cycle, Rio Grande do Sul, Brazil, from August/2011 to August/2012. (A) Off-season period. (B) Growing period. (C) Post-harvest period. Species abbreviations and crop age follow legend of figure 2.
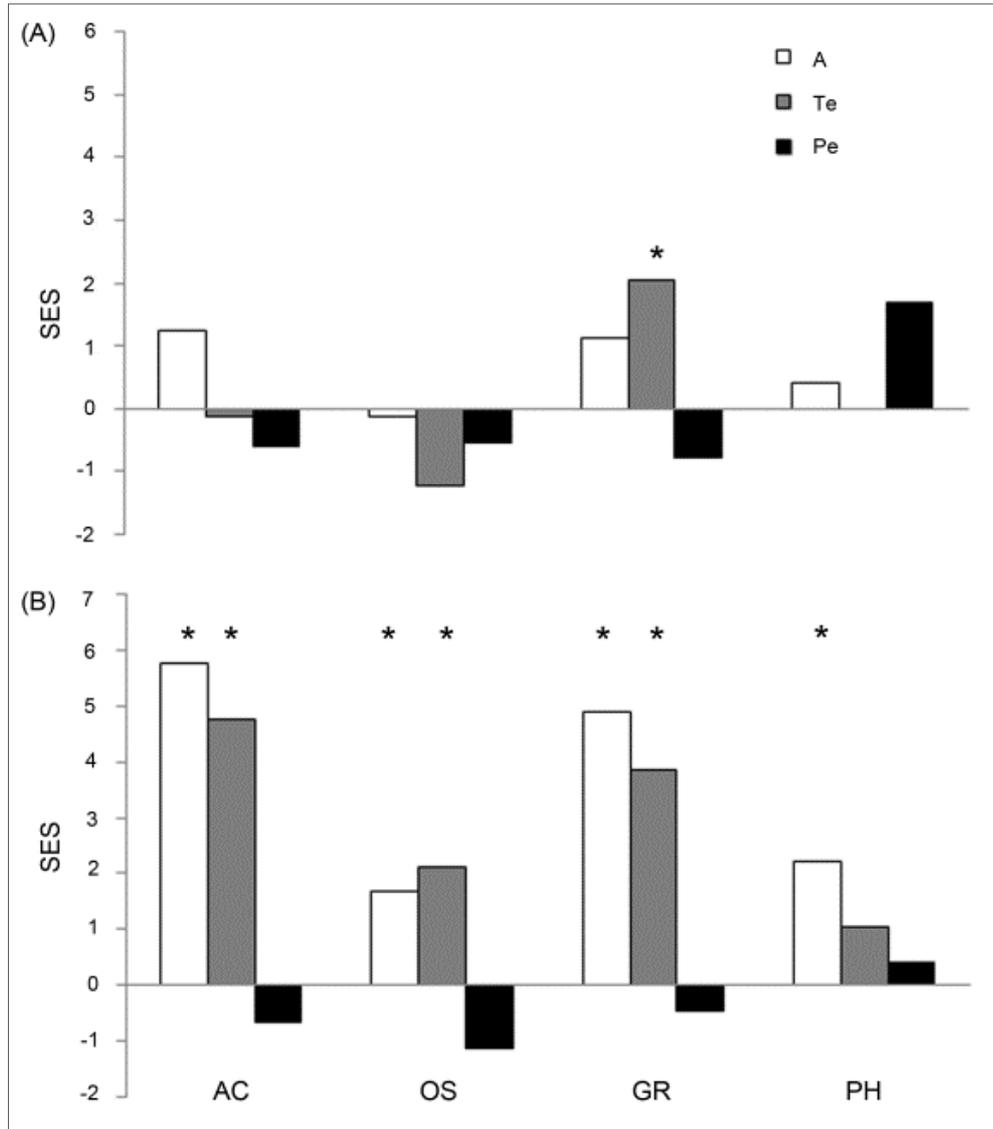
Figure 4. Variation in standardized effect size (SES) among the anuran guilds in different periods of rice cultivation (A), and among the anuran guilds in different crop ages in each cultivation period (B). Guilds: A= all species, Pe= perches species Te= terrestrial species. Cultivation cycle: AC= all cycle, OS= off-season, GR= rice growing, PH= post-harvest. Significant effect size are indicated by asterisk.

3.8 TABLES

Table 1. Anuran species found in a rice paddy area with different land use histories, Rio Grande do Sul, Brazil, across rice cultivation cycle (from August 2011 to August 2012). Short-term: 03 years of cultivation, Mid-term: 10 years of cultivation, Long-term: 20 years of cultivation. Ab: abundance, F: occurrence frequency, A: aquatic, T: terrestrial, Pe: perches

| Species	Guild	Natural ponds		Short-term rice		Mid-term rice		Long-term rice	
		Ab	F (%)	Ab	F (%)	Ab	F (%)	Ab	F (%)
Bufonidae									
Rhinella dorbignyi (Duméril & Bibron, 1841)	T	02	33.3	02	33.3	01	33.3	11	100
Hylidae									
Dendropsophus sanborni (Schmidt, 1944)	Pe	112	100	52	100	36	100	12	66.6
Dendropsophus minutus (Peters, 1872)	Pe	09	100	04	33.3				
Hypsiboas pulchellus (Duméril & Bibron, 1841)	Pe	112	100	08	100	25	66.6	09	66.6
Pseudis minuta Günther, 1858	A	165	100	01	33.3	20	33.3	01	33.3
Scinax berthae (Barrio, 1862)	Pe	16	33.3					01	33.3
Scinax granulatus (Peters, 1871)	Pe	02	66.6			01	33.3		
Scinax squalirostris (A. Lutz, 1925)	Pe	68	100	04	66.6	15	33.3		
Leiuperidae									
Physalaemus biligonigerus (Cope, 1861 "1860")	T					20	33.3	16	100
Physalaemus cuvieri Fitzinger, 1826	T							03	33.3
Physalaemus henselii (Peters,1872)	T	02	33.3						
Pseudopaludicola falcipes (Hensel, 1867)	T	34	100	23	100	17	100	25	100
Leptodactylidae									
Leptodactylus gracilis (Duméril & Bibron, 1841)	T	02	33.3	11	100	22	100	04	33.3
Leptodactylus latinasus Jiménez de la Espada 1875	T			11	66.6	16	100	19	66.6
Leptodactylus latrans (Steffen, 1815)	T	19	100	07	100	10	66.6	05	66.6
Microhylidae									
Elachistocleis bicolor (Valenciennes in Guérin-Menéville,1838)	T	05	66.6	01	33.3	05	33.3	05	100

Table 2. PERMANOVA comparing anuran assemblages across natural ponds and rice fields with different cultivation ages (03,10, and 20 years), and across sampling occasions.

Comparison	R^2	df	F	p
Global comparison across all historic	0.512	3	2.907	**0.015**
Off-season	0.482	3	2.485	**0.033**
Growing	0.398	3	1.76	**0.03**
Post-harvest	0.363	3	1.517	0.164

Table 3. Results of co-occurrence analyses. Separate analyses were carried out for each guild across the whole cultivation cycle and for each cultivation period. Mean sim index: Mean simulated C-score from 5000 random runs. SES: standardized effect size.

Period	Class	C-score	Mean sim index	SES	p (obs ≤ exp)	p (obs ≥ exp)
Whole cycle	*Fixed-fixed*					
	Whole assemblage	4.515	4.408	1.25	0.918	0.095
	Perches	1.238	1.336	-0.60	0.418	0.757
	Terrestrial	5.143	5.165	-0.13	0.544	0.547
	Fixed-weighted					
	Whole assemblage	4.515	2.085	5.78	1	**<0.001**
	Perches	1.238	1.638	-0.67	0.274	0.754
	Terrestrial	5.143	1.821	4.75	1	**<0.001**
Off-season	*Fixed-fixed*					
	Whole assemblage	3.295	3.319	-0.14	0.49	0.552
	Perches	0.533	0.628	-0.55	0.664	1
	Terrestrial	5.200	5.520	-1.23	0.107	0.957
	Fixed-weighted					
	Whole assemblage	3.295	2.463	1.68	0.961	**0.042**
	Perches	0.533	1.159	-1.13	0.134	0.891
	Terrestrial	5.200	3.241	2.12	0.983	**0.02**
Growing	*Fixed-fixed*					
	Whole assemblage	5.718	5.600	1.11	0.875	0.146
	Perches	1.500	1.788	-0.78	0.435	1
	Terrestrial	6.607	6.257	2.03	0.968	**0.04**
	Fixed-weighted					
	Whole assemblage	5.718	2.969	4.91	1	**<0.001**
	Perches	1.500	2.00	-0.48	0.384	0.734
	Terrestrial	6.607	3.227	3.85	1	**<0.001**
Post-harvest	*Fixed-fixed*					
	Whole assemblage	4.467	4.305	0.42	0.733	0.321
	Perches	5.333	4.676	1.68	1	0.185
	Terrestrial	2.333	2.333	0	1	1
	Fixed-weighted					
	Whole assemblage	4.467	2.932	2.22	0.995	**0.006**
	Perches	5.333	4.521	0.41	0.663	0.361
	Terrestrial	2.333	1.619	1.05	1	0.368

Table 4. Measure of nestedness for anuran assemblages in a rice paddy area with different crop ages at southern Brazil. NODF: nestedness across all sites, NODF(q): nestedness of null model where presences are randomly assigned, but both row and column frequencies are maintained, p(q): significance of NODF based on this null model.

Order	Matrix	NODF	NODF(q)	p(q)
Frequency	Whole assemblage	67.39	67.86	0.465
	Perches	58.08	57.47	0.398
	Terrestrial	65.50	64.41	0.248
Historic	Whole assemblage	39.19	41.93	**0.004**
	Perches	50.50	50.09	0.903
	Terrestrial	15.88	14.00	**0.018**

4 CAPÍTULO 3

Este capítulo será submetido na forma de artigo científico ao periódico *Landscape Ecology*.

Anuran abundance in the Pampa wetlands: the role of local and regional factors

Leonardo F. B. Moreira [1,*]; Rafael G. Moura [2], and Leonardo Maltchik [1]

[1] Laboratório de Ecologia e Conservação de Ecossistemas Aquáticos, Universidade do Vale do Rio dos Sinos - UNISINOS, Av. Unisinos, 950, 93022-000, São Leopoldo, Brasil

[2] Laboratório de Ecologia de Peixes, Universidade do Vale do Rio dos Sinos - UNISINOS

* corresponding author: e-mail: leonardobm@gmail.com

Abstract

Land-uses in agricultural areas play an important role in amphibian conservation, since landscape changes have been recognized as a major cause of biodiversity lost. Here, we examined the relationship between anuran abundance and habitat features at different spatial scales in Pampa biome, southern Brazil. For this goal, we selected eleven local variables and nine land-cover uses surrounding 30 wetlands at four spatial scales: circular areas of 500, 1000, 3000 and 5000 m radii. Analyses evaluated support for a series of alternative models, designed to assess the scale at which habitat features influence three wide-spread anuran species. Results supported models that included both local and landscape variables. Species differed in association with hydroperiod and steppe vegetation. *Scinax squalirostris* abundance was best predicted by local, wetland-linked variables (hydroperiod), while *Hypsiboas pulchellus* abundance was associated with steppe vegetation in 3000 m buffer and locally with water quality and emergent vegetation. *Odontophrynus americanus* was positively associated with pioneer vegetation in 5000 m buffer. Our results highlighted the importance of developing conservation strategies based on multiple spatial scales. We propose that the incorporation of individual species-traits in the establishment of buffer zones, because anurans do not all respond to the same spatial scale.

Keywords grasslands; land use; amphibian conservation; southern Brazil

4.1 INTRODUCTION

Over the past two decades, numerous studies have indicated that amphibian populations are declining in many parts of the world. In spite of increased public consciousness and the importance of remnant amphibian populations and the factors responsible for their decline (Becker and Zamudio 2011; Eterovick et al. 2005), we still do not completely understand how these factors operate on the population level, whether acting alone or with others (Beebee and Griffiths 2005; Blaustein et al. 2010; Mann et al. 2009; Pimenta et al. 2005). Although the causes of population declines may vary from region to region and even within different populations of the same species (Blaustein et al. 2010), there is currently a consensus that species declines that occur at low elevations are most frequently associated with habitat loss or modification (Becker and Zamudio 2011; Cushman 2006; Gallant et al. 2007; Hero and Morrison 2004).

Agriculture counts for the largest portion of land use in most of Europe and North America (Green et al. 2005). Around one-third of Brazil is covered by agriculture, which is likely to increase as demand for food and biofuels increases (Ferreira et al. 2012; Sparovek et al. 2010). In this sense, the habitat loss associated with agricultural expansion is probably the principal cause of population declines (Gallant et al. 2007). As with other organisms, habitat loss affects amphibian species through reduced natural habitats, population isolation, inbreeding and factors associated with edge effects (changes in microclimates and interaction with exotic species) (Cushman 2006; Dixo et al. 2009; Ficetola and De Bernardi 2004; Machado et al. 2012; Watling et al. 2011). Wetlands in agricultural landscapes often accumulate pollutants (Mann et al. 2009) and have altered hydroperiods (Venne et al. 2012), which negatively affect amphibian populations. Moreover, agricultural activities operate on levels that vary from local to landscape and elicit different responses in communities depending on the ecological traits of the species present (Becker et al. 2010; Burel et al.

2004). Understanding the association between habitat use and the distribution of species associated with wetlands is critical to establishing conservation strategies.

An idea that has been considered important to amphibian conservation is habitat complementarity (Pope et al. 2000). Due to the biphasic life cycle of amphibians, the proximity of breeding habitats and terrestrial areas for foraging and dispersal is a key factor in the occupation of areas by amphibians with aquatic larvae (e.g.: Babbitt et al. 2009; Guerry and Hunter Jr 2002; Hocking and Semlitsch 2007). Many of the impacts caused by agricultural activities are related to the effects they have on matrix quality (Benton et al. 2003; Donald and Evans 2006). In landscapes altered by human activities, habitat split not only reduces population size and dispersal dynamics, but also negatively affects local amphibian community richness (Becker et al. 2007). In the literature, one of the most consistent relationships on a landscape level is the positive association between forest habitats and amphibian communities (Becker et al. 2007; Herrmann et al. 2005; Porej et al. 2004). However, most studies have taken place in areas that were originally covered by forests. In grasslands, the associated modifications of agricultural use do not seem to have a remarkable effect. Although they favor the occurrence of species with explosive reproduction, these changes indirectly affect or do not affect amphibian abundance (Gray et al. 2004; Venne et al. 2012). In this type of environment, direct results of agricultural intensification are primarily associated with local factors such as changes in the hydroperiod and introduction of fish (Beja and Alcazar 2003; Machado and Maltchik 2010; Richter-Boix et al. 2007).

The Pampa biome is characterized by a mosaic of grasslands interspersed with scrub savannas and gallery forests that extends through Uruguay, Argentina and southern Brazil (Miñarro and Billenca 2008; MMA 2011). According to WWF Conservation Science (Olson et al. 2001), four biogeographically similar ecoregions are recognized in the biome: Humid Pampa (NT0803), Semi-arid Pampa (NT0806), Southern Cone Mesopotamia Pampa

(NT0909) and Uruguayan Savanna (NT0710). These different formations are under different degrees of conservation due to the historical presence of livestock activities. Yet, agricultural and silvicultural practices have greatly expanded since the second half of the 20th century, which has lead to both local and landscape changes in the pampas (Miñarro and Billenca 2008). Land use changes in southern Brazil have been poorly documented compared to other regions of the country (Overbeck et al. 2007). About 50% of the original Brazilian Pampa remains (MMA 2011), and untouched natural grasslands and forests have been reduced by almost 90% (Cordeiro and Hasenack 2009). Additionally, less than 0.5% of this biome is protected by conservation units (Overbeck et al. 2007).

We examined the relationship between anuran abundance and habitat characteristics of the Pampa biome on five spatial scales. Our objectives were to: i) determine the abundance of three anuran species in 30 wetlands subject to fragmentation caused by agriculture and livestock; ii) determine at which spatial scale species respond to habitat modifications; iii) examine the relationship between anuran abundance and habitat characteristics. To meet these objectives, we selected species that inhabit open areas and exhibit unspecialized reproductive modes (Maneyro and Carreira 2012). We assumed that specific elements of the landscape influenced species occurrence at different spatial scales and that land uses that did not greatly modify the grassland matrix could be seen as buffers to protect wetlands. We studied tadpoles instead of adults to determine species presence, and our analyses included environments that are effectively used for breeding activity.

4.2 METHODS

The study area is located in the southern half of Rio Grande do Sul (28.611° to 31.744°S ; 52.518° to 57.503°O), within the Pampa biome (Figure 1). Under ecoregion classification of WWF, this formation is within the Uruguayan savanna ecoregion. The

climate is subtropical with a wide temperature range, and while it may frost or snow in some regions, temperatures can reach 40°C in summer. Annual rainfall varies from 1200 to 1700 mm/year (Rossato 2011).

We selected 64 wetlands from Google Earth Pro 4.2.1 images. Since our focus was natural wetlands and the surrounding matrix, we used four selection criteria: distance between areas, presence of dykes, wetland size and distance from nearest road. Most of the areas in this region have been dug out for pisciculture or barred for livestock watering holes. Thus, we selected 50 areas that were at least 15 km from one another, did not have dykes, were less than 1 ha and within 200 m of the nearest road. Selecting areas close to roads allowed us to sample more locations and standardize distance to the road, which could affect anuran displacement (Carr and Fahrig 2001). We selected 30 amphibian sampling sites from the wetlands that met all four criteria.

The three species that we sampled are widely distributed in southern Brazil and neighboring countries and mainly inhabit open areas (Maneyro and Carreira 2012). *Hypsiboas pulchellus* breeds throughout the year and has benthic tadpoles with a long development time (three to six months). *Scinax squalirostris* and *Odontophrynus americanus* preferentially breed from September to April during rainy or warm periods. The tadpoles of both species go through metamorphosis after around three months, and the former is nektonic while the latter is benthonic.

We sampled tadpoles on four occasions between 2010 and 2012: twice at the end of winter (September/2010 and September/2011) and twice at the end of summer (March/2010 and March/2011). We measured tadpole abundance with the methodology proposed by Shaffer et al. (1994). We sampled five plots (50 x 50 cm) in each area at the time of sampling. All of the tadpoles within each plot were removed with a net (10 cm width). Each plot

sampling ended after 10 sweeps of the net without catching tadpoles. The tadpoles were anaesthetized with benzocaine, fixed in 10% formaldehyde and identified in the laboratory.

Twenty descriptors were used to evaluate the environmental characteristics of each wetland (Table 1). We measured physical and chemical water quality from surface water with a Horiba H-10 multiparameter water quality checker. We measured turbidity, conductivity, pH and reduction potential (ORP) at two points per sample in each wetland. We used the average of each parameter as the value for each area. Vegetation cover was classified in two classes: more than and less than 50% of the area covered by macrophytes. The different types of macrophytes (emergent, floating and submersed) were classified in five classes: $0 = $ no vegetation, $1 = < 5\%$, $2 = 5\text{-}25\%$, $3 = 25\text{-}75\%$, $4 = > 75\%$. The size of each wetland was calculated *in situ* by multiplying the greatest width by the greatest length of the water surface. Hydroperiod was measured by the number of sampling events with surface water.

We used geographical information system software (ArcGIS 10) to determine the proportion of cover in various land-class categories within 500, 1000, 3000 and 5000 m from the perimeter of each wetland. We selected these distances based on estimates of amphibian migration and dispersal (Semlitsch 2008). We used the soil use classification system developed by the Universidade Federal do Rio Grande do Sul at a scale of 1:250000 (Hasenack 2006). We transformed the classification scheme into raster (matrix) data with a 5 m resolution (pixel size 5 x 5) and applied the distances for landscape analysis. The classified images were cut into windows and Fragstats 4.1 was used to extract the area of each class from the processed images. We identified nine soil uses within 5000 m from the sample areas (Table 1).

Data analysis included two steps. We used a principal component analysis (PCA) to identify the principal variation gradients for variables at the local scale. In each PCA, we only used axes with eigenvalues greater than the average of all of the eigenvalues of the analysis

(Borcard et al. 2011). The variables that most contributed to each of the axes were identified through a circle of equilibrium contribution (Legendre and Legendre 1998). We then related the abundance of each species with the selected axes and landscape metrics as independent variables with a linear model regression using permutation tests(Wheeler 2010). We evaluated 15 potential models for each species based on the idea that abundance is influenced by a group of factors that operate on different spatial scales. Thus, the models were: only local variables; only landscape variables and combinations of variables on both scales, based on the literature (e.g. hydroperiod and vegetation area). We used AIC_c, a corrected version of Akaike information criteria, to identify which models received the greatest support from the data (Burnham and Anderson 2002). The best model has the lowest AIC_c, but it often is not supported much better than alternative models. So, we used Akaike weights (w_i) to evaluate the uncertainty of the model selection. We used the packages vegan (Oksanen et al. 2012) and ImPerm (Wheeler 2010) on R. 2.15 to run the analyses.

4.3 RESULTS

Out of 30 wetlands, we collected *Hypsiboas pulchellus* in 27, *Scinax squalirostris* in 19 and *Odontophrynus americanus* in 14. We captured an average of 12.7 (± 2.8 standard error) *H. pulchellus*, 2.4 (± 0.62) *S. squalirostris* and 2.1 (± 0.9) *O. americanus* individuals. On a local scale, the four components extracted by the PCA represented 61% of the original variance of the data. The first axis (Pc1) represented variations in acidity and plant cover of the water surface, and the second axis (Pc2) represented variations in area and altitude (Table 2). The third axis (Pc3) reflected the presence of solids in the water and was negatively correlated with percent emergent macrophytes, conductivity and turbidity. The fourth axis (Pc4) was positively correlated with hydroperiod and ORP.

Anuran abundance in Pampa wetlands was best described by a combination of landscape characteristics and local attributes, although this varied from species to species (Table 3). Local variables were only important for *H. pulchellus* and *S. squalirostris*. The best model for *H. pulchellus* incorporated local characteristics up to 3000 m. The w_i for this model was 0.35, which means that the model has 35% chance of being the best, based on the data. The species was more abundant in landscapes such as steppe and in wetlands with low conductivity, turbidity and percent emergent macrophytes. Models that incorporated local characteristics and percent steppe vegetation on scales of 500 and 1000 m also received substantial support for their validity ($\Delta AIC_c < 2.0$). Although the third axis on a local scale received little support from the data when analyzed in isolation ($\Delta AIC_c = 11.02$), it remarkably improved the ability of the models on a landscape scale, which alone had ΔAIC_c values between 8 and 12.

Local variables best explained *S. squalirostris* abundance. The best model positively associated abundance with the fourth axis of the PCA, which corresponded to variations in hydroperiod and ORP. However, the force of evidence for this model was low ($w_i = 0.179$). Models with landscape and local variables had substantial evidence on various scales (Table 3). In the second best model, *S. squalirostris* abundance was positively related with hydroperiod and silviculture at a scale of 5000 m.

Odontophrynus americanus abundance was best explained by habitat characteristics at a scale of 5000 m, and the model was well supported by the data ($w_i = 0.787$). At this scale, the species was positively associated with areas of pioneer vegetation and negatively associated with permanent bodies of water. Including areas with permanent bodies of water greatly improved the predictive ability of the model that only included pioneer vegetation (Table 3).

4.4 DISCUSSION

Our results stress the importance of conservation strategies being based on individual species characteristics. Anuran abundance was influenced by variables measured at both local and landscape levels, but the importance of each component varied according to the species. This supports the idea that factors on multiple spatial scales influence patterns of amphibian occurrence (Browne et al. 2009; Van Buskirk 2005; Pope et al. 2000). The spatial scales that affected *Hypsiboas pulchellus*, *Scinax squalirostris* and *Odontophrynus americanus* were consistent with movement estimates found in the literature (Eterovick et al. 2009; Semlitsch 2008), even though information about migration and dispersal processes are lacking for neotropical frogs.

Associations with hydroperiod and steppe vegetation around wetlands agree with what is known about the ecology of each species (Maneyro and Carreira 2012; Moreira et al. 2010). *Scinax squalirostris* abundance was more closely associated with local variables, but the opposite was observed for *H. pulchellus* and *O. americanus*. The pattern of support for the models for *S. squalirostris* also differed from the other two species. Most of the species were associated with one scale in particular (lowest AIC_c), and scales next to the focal scale showed weaker relationships with some support. *Scinax squalirostris* was strongly associated with both small (local) scales and large (5000 m) scales. Therefore, the occurrence of some species of anurans in Pampa wetlands would be more consistent with explanations at the metapopulation level (Marsh and Trenham 2001; Smith and Green 2005). Pope et al. (2000) suggested that the influence of a landscape characteristic on a specific location could be measured as the distance to the furthest habitat fragment that influences the population of a given location. Steppe vegetation was an important landscape component and explained the abundance of *H. pulchellus* and *S. squalirostris*, but the permeability of this landscape seems to be different for the two species. Matrix permeability is a complex concept that

encompasses, among other things, landscape composition and configuration, associated risk of predation and shelter availability (Cosentino et al. 2010; Dixo and Metzger 2010; Guerry and Hunter Jr 2002).

Although two species may be related to the same landscape trait, the combination with local characteristics could vary. *Hypsiboas pulchellus* was negatively associated with conductivity, turbidity and percent emergent macrophytes, while hydroperiod was the most important local characteristic to *S. squalirostris*. Conductivity reflects total dissolved solids, mineral particles or eutrophization, and sites with high conductivity could be the result of frequent disturbances or high evaporative rates and low water replacement (Welch and MacMahon 2005). Negative effects of conductivity are related to reduced rates of growth and survival in amphibians (Sanzo and Hecnar 2006; Snodgrass et al. 2008). The negative association between the abundance of *H. pulchellus* and emergent vegetation was unexpected since aquatic vegetation provides calling sites and refuge from potential predators (Kopp et al. 2006; Moreira et al. 2007). In open areas, emergent vegetation could play an important role in reducing the amount of water lost to evaporation by shading (Tsai et al. 2007), but plants could have higher rates of evapotranspiration than open water (Paulikonis and Schneider 2001). If the vegetation increases the rate of water loss, a larger biomass of vegetation could decrease the hydroperiod and be a disadvantage to species with long larval periods, such as *H. pulchellus*. Various species of amphibians are able to accelerate metamorphosis in response to a reduced volume of water (Wells 2007), but we cannot guarantee that this is the case of *H. pulchellus*.

Open areas should be a hostile matrix for species that are prone to desiccation and have low dispersal ability. However, the existence of seasonal flood regimes and fragments with higher herbaceous vegetation densities close to the ground could help explain some of the association observed in Pampa species. *Scinax squalirostris* is a species frequently found

in association with *Eryngium* spp. and other tall tussock grasses (*Andropogon*, *Cortaderia*, *Eryanthus*) (Maneyro and Carreira 2012; Peltzer et al. 2006). Water and dew that accumulate on the plants' leaves and branches may help to retain humidity and provide shelter to frogs during the day. Dispersing individuals may not be able to penetrate forested landscapes without these herbaceous components, which results in increased abundance in areas surrounded by silviculture. Exotic tree plantations alter the hydrologic regime of wetlands and inhibit the growth of native plants through allelopathic effects (Machado et al. 2012; Rolon et al. 2011).

Variations in anuran reproductive patterns are mainly related to seasonal variations and the hydroperiod of wetlands (Canavero et al. 2008; Wells 2007). The variables that best explain the abundance of *S. squalirostris* and *O. americanus* (hydroperiod and pioneer vegetation, respectively) could be seen as a reflex of the reproductive pattern of the species. In subtropical environments, species with prolonged reproduction depend on longer hydroperiods and higher temperatures. Species that have explosive reproduction, like *O. americanus*, are associated with heavy rain or floods. In the Pampa, areas with pioneer vegetation are mainly located in river floodplains (Cordeiro and Hasenack 2009), which is an environment that favors species with explosive reproduction. Regarding the spatial scale, our result was not unexpected since *O. americanus* burrows into the ground during unfavorable conditions and it's skin forms a cocoon to help reduce water loss (Wells 2007).

We were unable to determine whether the patterns that we found are the result of adult migration between groups of reproductive environments or a reflection of juvenile dispersal to areas far from the natal wetland. Adult philopatry to breeding sites has been observed in anurans, and the intensity of this philopatry varies from species to species and according to the regional distribution of wetlands (Smith and Green 2005). Amphibians that reproduce in temporary environments are much more likely to move between breeding sites because of

annual fluctuations of suitable breeding areas. The occurrence of species with high vagility would be favored in wetlands in initial successional stages (Semlitsch 2008), which are common in open areas of Pampa. Thus, the landscape could be reflecting the availability and characteristics of the habitats used by adults. This interpretation supports the idea that a landscape influence occurs even in the absence of metapopulation processes (Van Buskirk 2005). The landscape would influence the performance of individuals that are not dispersing among populations, and should not be considered a large scale mechanism.

Numerous studies have shown that juvenile dispersal is likely more common than adult dispersion in many species of amphibians (Guerry and Hunter Jr 2002; Palo et al. 2004; Wells 2007). Juveniles of most species have major physiological and morphological limitations (water loss/ reduced locomotor capacity) which limit movement over great distances. Therefore, interspecific differences in habitat needs will play an important role in responses to habitat elements (Eterovick et al. 2009; Ficetola et al. 2009). Semlitsch (2008) suggested that juvenile dispersal is divided into various discrete events which allows individuals to cross greater distances. We believe that this model adequately explains the relationship of the abundance of *S. squalirostris* and *O. americanus* with large spatial scales since these species metamorphose a small size (~10mm).

Although the Pampa biome has a notable presence of agro-pastoral activities, these land uses were not well supported by the data in our models. Intensely modified agricultural fields and habitat modifications from livestock practices have been implicated as causing lower amphibian richness and abundance in agricultural landscapes (Babbitt et al. 2009; Brum et al. 2013; Ficetola et al. 2009; Peltzer et al. 2006). However, extensive livestock and family agriculture are probably more compatible with maintaining diversity than many other potential uses of these private lands in the Pampa (i.e. silviculture, mining). Many ranches in the region are environmentally sustainable and retain a relatively large portion of natural area

and associated species. The conversion of agro-pastoral areas to silviculture (*Eucalyptus* spp., *Pinus* spp.) has been encouraged by public policies in southern Brazil in spite of the profound effects on the landscape physiognomy and species composition (Machado et al. 2012). In this context, agro-pastoral landscapes (under certain circumstances) could be an important component in conservation planning and the development of management concepts and practices that help maintain biodiversity in these areas is an important component in amphibian conservation planning.

In conclusion, we demonstrated that even wide-distributed and generalist species differ remarkably regarding the spatial scales that affect their abundances. Amphibian conservation strategies have focused on buffers zones around wetlands (Ficetola et al. 2009). Therefore, we stress the importance of incorporating individual species traits into the planning of these areas. Our study contributes as an important tool for guiding conservation projects in grassland biomes.

4.5 ACKNOWLEDGMENTS

4.6 REFERENCES

Babbitt KJ, Baber MJ, Childers DL, Hocking D (2009) Influence of agricultural upland habitat type on larval anuran assemblages in seasonally inundated wetlands. Wetlands 29:294–301

Becker CG, Fonseca CR, Haddad CFB, et al. (2007) Habitat split and the global decline of amphibians. Science 318:1775–1777

Becker CG, Loyola RD, Haddad CFB, Zamudio KR (2010) Integrating species life-history traits and patterns of deforestation in amphibian conservation planning. Divers Distrib 16:10–19

Becker CG, Zamudio KR (2011) Tropical amphibian populations experience higher disease risk in natural habitats. Proc Natl Acad Sci U S A 108:9893–9898

Beebee TJC, Griffiths R a. (2005) The amphibian decline crisis: a watershed for conservation biology? Biol Conserv 125:271–285

Beja P, Alcazar R (2003) Conservation of Mediterranean temporary ponds under agricultural intensification: an evaluation using amphibians. Biol Conserv 114:317–326

Benton TG, Vickery JA, Wilson JD (2003) Farmland biodiversity: is habitat heterogeneity the key? Trends Ecol Evol 18:182–188

Blaustein AR, Walls SC, Bancroft B a., et al. (2010) Direct and indirect effects of climate change on amphibian populations. Diversity 2:281–313

Borcard D, Gillet F, Legendre P (2011) Numerical ecology with R. Springer, New York

Browne CL, Paszkowski C a., Lee Foote A., et al. (2009) The relationship of amphibian abundance to habitat features across spatial scales in the Boreal Plains. Ecoscience 16:209–223

Brum FT, Gonçalves LO, Cappelatti L, et al. (2013) Land use explains the distribution of threatened new world amphibians better than climate. PloS one 8:e60742.

Burel F, Butet A, Delettre YR, Millàn de la Peña N (2004) Differential response of selected taxa to landscape context and agricultural intensification. Landsc Urban Plan 67:195–204

Burnham KP, Anderson DR (2002) Model selection and multi-model inference: a practical information-theoretic approach, Springer, New York

Canavero A, Arim M, Naya DE, et al. (2008) Calling activity patterns in an anuran assemblage: the role of seasonal trends and weather determinants. North-West J Zool 4:29–41

Carr LW, Fahrig L (2001) Effect of road traffic on two amphibian species of differing vagility. Conserv Biol 15:1071–1078

Cordeiro JLP, Hasenack H (2009) Cobertura vegetal atual do Rio Grande do Sul. In: Pillar VD, Müller SC, Castilhos ZMS, Jacques AVA (eds) Campos sulinos: conservação e uso sustentável da biodiversidade. Ministério do Meio Ambiente, Brasília, pp 285–299

Cosentino BJ, Schooley RL, Phillips C a. (2010) Connectivity of agroecosystems: dispersal costs can vary among crops. Landsc Ecol 26:371–379

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biol Conserv 128:231–240

Dixo M, Metzger JP (2010) The matrix-tolerance hypothesis: an empirical test with frogs in the Atlantic Forest. Biodivers Conserv 19:3059–3071

Dixo M, Metzger JP, Morgante JS, Zamudio KR (2009) Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. Biol Conserv 142:1560–1569

Donald PF, Evans AD (2006) Habitat connectivity and matrix restoration: the wider implications of agri-environment schemes. J Appl Ecol 43:209–218

Eterovick PC, Carolina A, Queiroz O De, et al. (2005) Amphibian Declines in Brazil: an overview. Biotropica 37:166–179

Eterovick PC, Yazbeck GM, Dergam JA, Kalapothakis E (2009) Small scale population structure in the treefrog *Bokermannohyla saxicola* (Bokermann , 1964) (Anura , Hylidae). South Am J Herpetol 4:235–244

Ferreira J, Pardini R, Metzger JP, et al. (2012) Towards environmentally sustainable agriculture in Brazil: challenges and opportunities for applied ecological research. J Appl Ecol 49:535–541

Ficetola GF, De Bernardi F (2004) Amphibians in a human-dominated landscape: the community structure is related to habitat features and isolation. Biol Conserv 119:219–230

Ficetola GF, Padoa-Schioppa E, De Bernardi F (2009) Influence of landscape elements in riparian buffers on the conservation of semiaquatic amphibians. Conserv Biol 23:114–123

Gallant AL, Klaver RW, Casper GS, Lanoo MJ (2007) Global rates of habitat loss and implications for amphibian conservation. Copeia 2007:967–979

Gray MJ, Smith LM, Brenes R (2004) Effects of agricultural cultivation on demographics of southern high plains amphibians. Conserv Biol 18:1368–1377

Green RE, Cornell SJ, Scharlemann JPW, Balmford A (2005) Farming and the fate of wild nature. Science 307:550–555

Guerry AD, Hunter Jr ML (2002) Amphibian distributions in a landscape of forests and agriculture: an examination of landscape composition and configuration. Conserv Biol 16:745–754

Hasenack H (2006) Base cartográfica digital do Rio Grande do Sul 1:250000. Editora UFRGS, Porto Alegre

Hero JM, Morrison C (2004) Frog declines in Australia: global implications. Herpetol J 14:175–186

Herrmann HL, Babbitt KJKJ, Baber MJMJ, Congalton RG (2005) Effects of landscape characteristics on amphibian distribution in a forest-dominated landscape. Biol Conserv 123:139–149

Hocking DJ, Semlitsch RD (2007) Effects of timber harvest on breeding-site selection by gray treefrogs (*Hyla versicolor*). Biol Conserv 138:506–513

Kopp K, Wachlevski M, Eterovick PC (2006) Environmental complexity reduces tadpole predation by water bugs. Can J Zool 84:136–140

Legendre P, Legendre L (1998) Numerical Ecology. Elsevier Scientific Publishing Company, Amsterdam,Netherlands

Machado IF, Maltchik L (2010) Can management practices in rice fields contribute to amphibian conservation in southern Brazilian wetlands? Aquat Conserv 46:39–46

Machado IF, Moreira LFB, Maltchik L (2012) Effects of pine invasion on anurans assemblage in southern Brazil coastal ponds. Amphibia-Reptilia 33:227–237

Maneyro R, Carreira S (2012) Guía de anfíbios del Urugay. Ediciones de la Fuga, Montevideo

Mann RM, Hyne R V, Choung CB, Wilson SP (2009) Amphibians and agricultural chemicals: review of the risks in a complex environment. Environ Pollut 157:2903–2927

Marsh DM, Trenham PC (2001) Metapopulation dynamics and amphibian conservation. Conserv Biol 15:40–49

McCauley SJ, Bouchard SS, Farina BJ, et al. (2000) Energetic dynamics and anuran breeding phenology: insights from a dynamic game. Behav Ecol 11:429–436

Miñarro F, Billenca D (2008) The conservation status of temperate grasslands in central Argentina. Fundación Vida Silvestre Argentina, Buenos Aires, Argentina

MMA (2011) Ministério do Meio Ambiente. monitoramento do desmatamento nos biomas brasileiros por satélite acordo de cooperação técnica mma / ibama monitoramento do bioma pampa. Available from http://www.mma.gov.br/estruturas/sbf_chm_rbbio/_arquivos/relatrio_tcnico_monitoramento_pampa_2008_2009_72.pdf (acessed April 2013)

Moreira LFB, Machado IF, Garcia TV, Maltchik L (2010) Factors influencing anuran distribution in coastal dune wetlands in southern Brazil. J Nat Hist 44:1493–1507

Moreira LFB, Machado IF, Lace ARGM, Maltchik L (2007) Calling period and reproductive modes in an anuran community of a temporary pond in southern Brazil. South Am J Herpetol 2:129–135

Oksanen J, Blanchet FG, Kindt R, et al. (2012) vegan: Community Ecology Package. Available from http://CRAN.R-project.org/package=vegan (acessed March 2013)

Olson DM, Dinerstein E, Wikramanayake ED, et al. (2001) Terrestrial ecoregions of the world: a new map of life on Earth. BioScience 51:933–938

Overbeck G, Muller S, Fidelis a, et al. (2007) Brazil's neglected biome: the South Brazilian Campos. Perspect Plant Ecol Evol Syst 9:101–116

Palo JU, Lesbarrères D, Schmeller DS, et al. (2004) Microsatellite marker data suggest sex-biased dispersal in the common frog *Rana temporaria*. Mol Ecol 13:2865–2869

Paulikonis N, Schneider R (2001) Temporal patterns in evapotranspiration from lysimeters with three common wetland plant species in the eastern United States. Aquat Bot 71:35–46

Peltzer PM, Lajmanovich RC, Attademo AM, Beltzer AH (2006) Diversity of anurans across agricultural ponds in Argentina. Biodivers Conserv 15:3499–3513

Pimenta BVS, Haddad CFB, Nascimento LB, et al. (2005) Comment on "Status and trends of amphibian declines and extinctions worldwide". Science 309:1999

Pope SE, Fahrig L, Merriam HG (2000) Landscape complementation and metapopulation effects on leopard frog populations. Ecology 81:2498–2508

Porej D, Micacchion M, Hetherington TE (2004) Core terrestrial habitat for conservation of local populations of salamanders and wood frogs in agricultural landscapes. Biol Conserv 120:399–409

Richter-Boix A, Llorente GA, Montori A (2007) Structure and dynamics of an amphibian metacommunity in two regions. J Anim Ecol 76:607–618

Rolon AS, Rocha O, Maltchik L (2011) Does pine occurrence influence the macrophyte assemblage in southern Brazil ponds? Hydrobiologia 675:157–165

Rossato MS (2011) Os climas do Rio Grande do Sul: variabilidade, tendência e tipologia. Thesis, Universidade Federal do Rio Grande do Sul

Sanzo D, Hecnar SJ (2006) Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica*). Environ pollut 140:247–56

Semlitsch RD (2008) Differentiating migration and dispersal processes for pond-breeding amphibians. J Wildl Manage 72:260–267

Shaffer HB, Alford RA, Woodward BD, et al. (1994) Standard techniques for inventory and monitoring: quantitative sampling of amphibian larvae. In: Heyer WR, Donelly MA, McDiarmid RW, et al. (eds) Measuring and monitoring biological diversity. standard methods for amphibians. Smithsonian Institution Press, Washington, USA, pp 130–141

Smith MA, Green DM (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography 1:110–128

Snodgrass JW, Casey RE, Joseph D, Simon J a (2008) Microcosm investigations of stormwater pond sediment toxicity to embryonic and larval amphibians: variation in sensitivity among species. Environ pollut 154:291–297

Sparovek G, Berndes G, Klug ILF, Barretto AGOP (2010) Brazilian agriculture and environmental legislation: status and future challenges. Environ Sci Technol 44:6046–53

Tsai J-S, Venne LS, McMurry ST, Smith LM (2007) Influences of land use and wetland characteristics on water loss rates and hydroperiods of playas in the southern High Plains, USA. Wetlands 27:683–692

Van Buskirk J (2005) Local and landscape influence on amphibian occurrence. Ecology 86:1936–1947

Venne LS, Tsai J-S, Cox SB, et al. (2012) Amphibian community richness in cropland and grassland playas in the southern High Plains, USA. Wetlands 32:619–629

Watling JI, Hickman CR, Orrock JL (2011) Invasive shrub alters native forest amphibian communities. Biol Conserv 144:2597–2601

Welch NE, MacMahon JA (2005) Identifying habitat variables important to the rare Columbia Spotted Frog in Utah (U.S.A.): an information-theoretic approach. Conserv Biol 19:473–481

Wells KD (2007) The ecology and behavior of amphibians. The University of Chicago Press, London

Wheeler B (2010) lmPerm: Permutation tests for linear models. Available from http://cran.r-project.org/package=lmPerm (acessed March 2013)

4.7 FIGURES
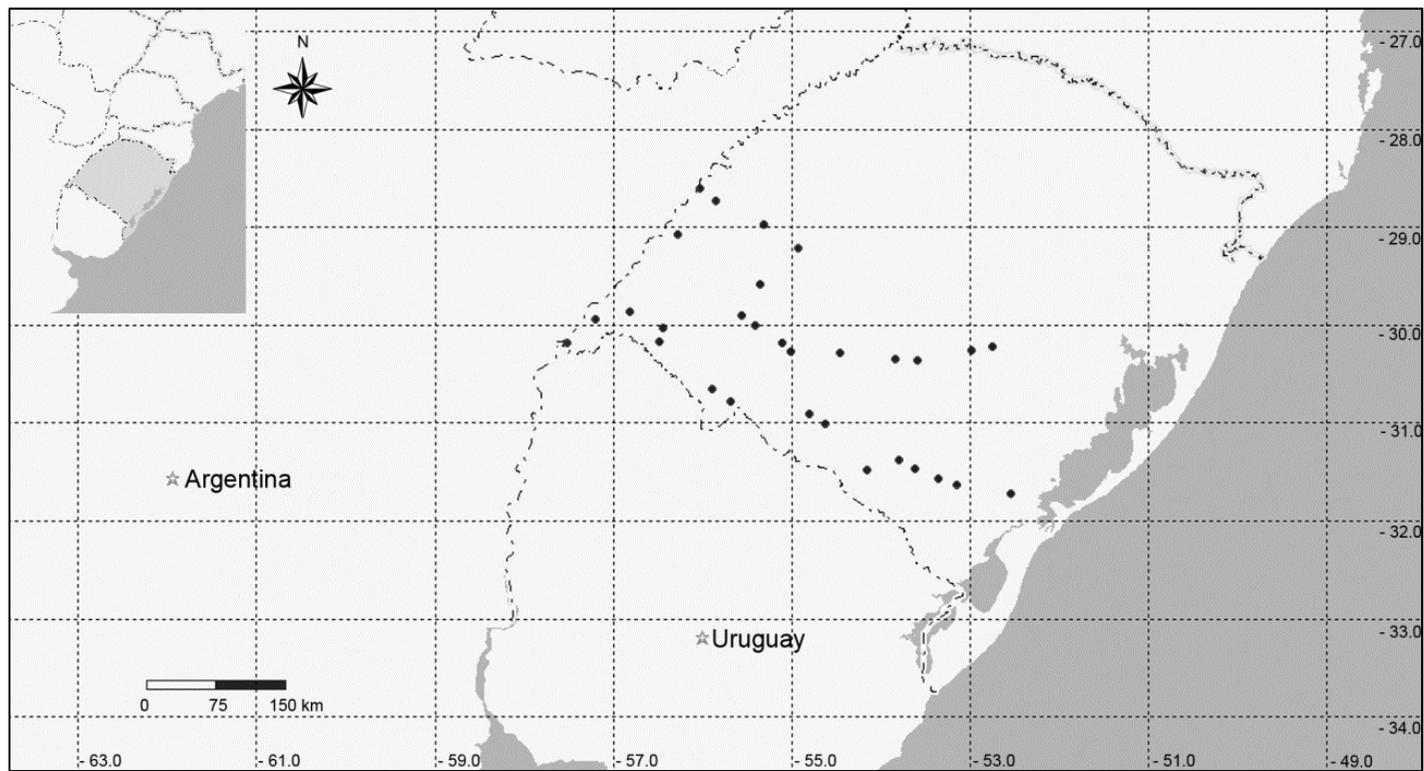
Figure 1. Map of 30 wetlands studied in the Pampa biome, Rio Grande do Sul, Brazil.

4.8 TABLES

Table 1. Environmental variables over 30 study wetlands in biome Pampa, Brazil

Scale	Variables	Mean (range)
Local	pH	6.4 (5.8-6.8)
	Turbidity	170(31.5-540) NTU
	Conductivity	7.3(2-19.5) µS/cm
	Redox potential (ORP)	368.8 (285-456) mV
	Vegetation cover	0.5 (0 = <50%, 1= > 50%)
	Submersed macrophytes [**]	1.4 (0- 3)
	Emergent macrophytes [**]	1.87(0-4)
	Floating macrophytes [**]	0.97(0-4)
	Area	0.30 (0.01- 1) ha
	Altitude	159 (47-380) m
	Hydroperiod	2.75(1-4) sampling
Landscape[*]	Agricultural areas	17.5 (0-65.8)
	Livestock ranches	32.8 (0-100)
	Water	2.3 (0-11)
	Steppes	13.4 (0-97)
	Seasonal forests	3.8 (0-13.7)
	Pioneering vegetation	0.3 (0-3)
	Silviculture	0.6 (0-9.9)
	Steppic savannas	28.2 (0-97.4)
	Urban areas	0.9 (0-20)

*: % cover within a 5000 m radius of each wetland. **: 0 = absence, 1 = < 5 %, 2 = 5-25 %, 3 = 25-75 %, 4= >75 %

Table 2. Principal component analysis using local variables. The variables listed have a high correlation (> 0.6) with each principal component and have positive correlations unless marked with negative sign (-)

Axis	Variable	% Explained
Pc1	pH	22
	- Vegetation cover	
	- Floating macrophytes	
Pc2	Area	15
	- Altitude	
Pc3	- Submersed macrophytes	13
	- Conductivity	
	- Turbidity	
Pc4	Hydroperiod	11
	ORP	

Table 3. Model selection results relating environmental characteristics at five increasing scales against abundance of the three anuran species, in Pampa biome

Species	Scale	Model variables	Coef. direction	ΔAIC_c	w_i	$R^{2\,*}$
Hypsiboas pulchellus	500 m/Local	Pc3;Steppe	+;+	0.94	0.218	
	1000 m/Local	Pc3;Steppe	+;+	0.33	0.295	
	3000 m/Local	Pc3;Steppe	+;+	0.00	0.349	0.452[a]
	5000 m/Local	Pc3;Steppe	+;+	2.26	0.112	
Scinax squalirostris	Local	Pc4	+	0.00	0.179	0.111[b]
	500 m	Steppe	-	2.29	0.057	
	1000 m	Steppe	-	1.71	0.076	
	1000 m/Local	Pc4;Water	+;+	1.17	0.099	
	3000 m	Steppe	-	1.57	0.081	
	3000 m/Local	Pc4;Livestock	+;+	1.58	0.081	
	5000 m/Local	Pc4;Pioneer veg	+;-	1.68	0.077	
		Pc4;Urban areas	++	1.15	0.100	
		Pc4;Silviculture	+;+	0.21	0.162	
Odontophrynus americanus	5000 m	Pioneer veg	+	6.10	0.040	
		Pioneer veg; Water	+;-	0.00	0.787	0.44[a]

Only models with lowest ΔAICC are showed; wi: Akaike weights; * Result of best model; a: p<0.001; b:p<0.05

5 CONSIDERAÇÕES FINAIS

Os resultados obtidos na minha tese reforçam a necessidade de se incorporar características individuais das espécies na avaliação dos impactos da intensificação agrícola. Diferentes componentes da paisagem e fatores históricos do manejo da terra podem atuar sobre a distribuição dos anfíbios em áreas do Pampa. Como vimos aqui, mesmo anuros tolerantes a alterações antrópicas e que exibem modos de reprodução não especializados têm sua ocorrência restrita, dentro de agroecossistemas.

A expansão de terras cultivadas e o grande interesse na perda de biodiversidade têm fomentado pesquisas que integrem o manejo de áreas agrícolas com vista à conservação da biodiversidade. No primeiro capítulo, eu encontrei evidências de que arrozais orgânicos abrigam uma diversidade maior de anuros, em comparação a lavouras não orgânicas, além de uma composição diferenciada. Meus resultados insinuam que estas práticas agrícolas poderiam beneficiar a fauna de anfíbios, assim seus impactos sobre populações de anuros constituiriam uma área de frutíferas investigações futuras. Contudo, atribuir esse resultado à alguma prática específica do manejo orgânico é complicado. Essa incerteza reside no fato de que paisagens agrícolas estão em constante estado de fluxo. Rotação de culturas e mudanças no uso e manejo da terra; diferenças no uso de agroquímicos, formulações e taxas de aplicação; variação regional na estrutura da comunidade; e interações com agentes ambientais adversos, como mudanças globais no clima. Todos esses fatores criam dificuldades na hora de se atribuir uma relação de causa e efeito.

Eu também demonstrei que o histórico da área pode modificar a estrutura das comunidades de anuros que utilizam arrozais. A permeabilidade da matriz agrícola e suas potenciais consequências na dispersão das espécies parecem ser as principais restrições a ocorrência das espécies, nesses agroecossistemas. Enquanto a idade da lavoura é claramente importante, os mecanismos por trás dessas respostas necessitam ser identificados. Em particular, nós precisamos estabelecer porque espécies terrestres mostraram padrões de segregação condicionados ao histórico da área. Apesar da noção de que arrozais contribuem para a conservação de organismos que utilizam áreas inundáveis; campos de arroz irrigado não podem ser considerados como equivalentes de áreas úmidas naturais, por que eles não fornecem condições adequadas para todo o conjunto de espécies que utilizam essas áreas sazonalmente inundadas.

No terceiro e último capítulo, eu construí alguns modelos contendo informação sobre processos ambientais e espaciais em diferentes escalas para predizer a abundância de larvas de

anuros. A abundância de girinos foi influenciada por variáveis medidas em escala local e também de paisagem, contudo a importância da contribuição de cada componente variou conforme a espécie. Eu mostrei que a utilização de sítios reprodutivos por anuros está associada com a presença de matriz campestre (estepe ou vegetação pioneira) e que a abundância de algumas espécies está associada com preferências locais, como hidroperíodo e química da água. Como na metade sul do Rio Grande do Sul a conversão de paisagens agropastoris para monoculturas florestais exóticas continua, a manutenção de paisagens pecuárias e com agricultura familiar (sobre certas condições) podem ser um importante componente de planejamentos de conservação.

De maneira geral, considerando os três capítulos aqui apresentados, uma diretriz para a conservação de anfíbios em áreas agrícolas do sul do Brasil é a necessidade de foco em políticas de uso da terra. Em minha opinião, futuros estudos deveriam comparar as respostas de espécies diferentes para os mesmos tipos de matriz ou manejo, a fim de determinar se existem padrões gerais para espécies compartilhando atributos biológicos comuns, e a extensão das respostas espécie-específicas.