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A INFLUÊNCIA DE GRADIENTES AMBIENTAIS E DA RADIAÇÃO ULTRAVIOLETA SOBRE COMUNIDADES DE ANUROS DO EXTREMO SUL DA MATA ATLÂNTICA

TESE DE DOUTORADO

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Santa Maria, RS, Brasil

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Victor Mendes Lipinski

Tese apresentada ao Programa de Pós-Graduação em Biodiversidade Animal, da Universidade Federal de Santa Maria, como requisito parcial para obtenção do título de Doutor em Ciências Biológicas – Área Biodiversidade Animal.

Orientador: Prof. Dr. Tiago Gomes dos Santos

Co-Orientador: Prof. Dr. André Passaglia Schuch

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elaborada por

Victor Mendes Lipinski

como requisito parcial para obtenção do título de Doutor em Ciências Biológicas – Área Biodiversidade Animal

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“The world is not in your books and maps, it’s out there”

(Gandalf, the grey)

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Biodiversidade Animal
Universidade Federal de Santa Maria

A INFLUÊNCIA DE GRADIENTES AMBIENTAIS E DA RADIAÇÃO ULTRAVIOLETA SOBRE COMUNIDADES DE ANUROS DO EXTREMO SUL DA MATA ATLÂNTICA

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Data e Local de Defesa: Santa Maria, 16 de fevereiro de 2017.

Os anfíbios estão entre os grupos de vertebrados mais ameaçados do planeta, principalmente devido à atividade antrópica e aos efeitos secundários decorrentes destas atividades. Desta forma, no presente trabalho nós buscamos avaliar como a modificação da paisagem afeta as comunidades de anuros em uma região que historicamente sofre pressão de conversão do habitat florestal em paisagem agrícola. Avaliamos como a radiação UVA e UVB afetam a sobrevivência e taxas de mortalidade de uma espécie de anuro endêmica da Mata Atlântica. Realizamos ensaios em laboratório replicando as doses diárias de radiação ultravioleta recebidas naturalmente, bem como simulando o aumento da incidência da mesma. Demonstramos que o aumento, ainda que pequeno, da radiação sobre as desovas é capaz de induzir morte e mutações nos anuros. Avaliamos também a forma com que as comunidades de anuros, em poças no interior da mata preservada e na matriz agrícola, são estruturadas, utilizando abordagem em análise de aninhamento e partição da diversidade beta. Demonstramos que na comunidade existe substituição de espécies ao longo do gradiente estudado. Dentro do mesmo gradiente, testamos como se comporta a diversidade funcional dos estágios larvais dos anuros a medida que aumenta a distância entre os sítios de reprodução e a atividade agrícola. Assim demonstramos que ainda que se encontrem em uma unidade de conservação, os estágios larvais de anuros do Parque Estadual do Turvo apresentam um baixo número de grupos funcionais o que põe em risco a conservação das espécies presentes. Testamos também de que forma a diversidade funcional, taxonômica e filogenética das comunidades pode se alterar neste gradiente, analisando quatro matrizes de dados, contendo os traços funcionas, dados ambientais, dados de abundância e uma matriz filogenética. Desta forma, registramos que a perda de espécies é responsável pelo aumento da diversidade filogenética em poças na matriz agrícola, um padrão que não ocorre em poças dentro da mata preservada. O presente trabalho demonstra que mesmo as áreas protegidas por lei sofrem os efeitos diretos e indiretos da atividade antrópica que ocorre no entorno, além disso, torna evidente que a flexibilização das leis ambientais que estão sendo propostas pode causar severos impactos na fauna silvestre e na estabilidade dos ecossistemas.

ABSTRACT

Doctorate Thesis
Post-Graduation in Animal Biodiversity
Universidade Federal de Santa Maria

THE INFLUENCE OF ENVIRONMENTAL GRADIENTS AND OF THE ULTRAVIOLET RADIATION ON ANURAN COMMUNITIES AT SOUTHERNMOST ATLANTIC RAINFOREST

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CO-ADVISOR: ANDRÉ PASSAGLIA SCHUCH
Date and place: Santa Maria, February, 16, 2017.

Amphibians are among the most threatened vertebrate groups on the planet, mainly due to anthropic activity and the side effects of these activities. Thus, in the present work we evaluated how the modification of the landscape affects the communities of anurans in a region that historically undergoes habitat conversion pressure of forest into agricultural landscape. We evaluated how UVA and UVB radiation affects the survival and mortality rates of an endemic species of the Atlantic Forest. We carried laboratory tests replicating daily doses of naturally received ultraviolet radiation , such as the doses simulating the increased incidence of the same. We demonstrated that the increase, even a small, of the radiation on the spawning is able to induce death and mutations in the anurans. In addition, we also evaluated how the anuran communities, in ponds both inside the preserved forest and in the agricultural matrix, are structured, using a nesting analysis and beta diversity partition approaches. In this way, we demonstrate through the community that there is a species substitution along the gradient studied. Within the same gradient, we tested how the functional diversity of the larval stages of the anurans behaves according increase the distance between breeding ponds and the agricultural activity. Thus, we show that although they are in a conservation unit, the larval stages of anurans of the Parque Estadual do Turvo present a low number of functional groups, which endangers the conservation of the present species. We also tested how the functional, taxonomic and phylogenetic diversity of the communities can change in this gradient, analyzing four data matrices, containing functional traits, environmental data, abundance data and a phylogenetic matrix. In this way, we recorded that the loss of species is responsible for the increase of phylogenetic diversity within agricultural matrix, a pattern that does not occur within the preserved forest. The present work shows that even the protected areas suffer the direct and indirect effects of the anthropic activity that occurs in the surroundings, besides, it makes evident that the flexibilization of the environmental laws that are being proposed can cause severe negative impacts on the fauna and the stability ecosystems.

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Introdução Geral

Ecologia e comunidades ecológicas: breve histórico e algumas definições.

A ecologia nem sempre foi tida como uma disciplina científica, já que por muitos anos a ecologia era apenas um meio de descrever a natureza. No entanto, com o passar do tempo a ecologia estabeleceu-se como uma ciência separada das demais (através da demonstração científica de que biologia e ecologia não são sinônimos, uma ideia incialmente definida (conceitualizada) por Haeckel (1866), onde *ecologia* é o estudo científico das interações entre os organismos e seu ambiente. Essas primeiras ideias e bases científicas já podiam ser observadas antes, em trabalhos como o de Charles Darwin publicado em 1859 (Darwin 1859), que abriram, de certa forma, um caminho para estudos sistemáticos das relações entre os organismos vivos e os locais em que habitam. Segundo Michael Begon e colaboradores (Begon et al. 2007), essa (a versão de Haeckel) seria uma definição ainda simplista, que veio a ser substituída ou ampliada por visões mais complexas.

Alguns anos depois, pesquisadores como John S. Burdon-Sanderson (1893) e Charles Elton (1927) foram de grande influência na criação de um conceito mais completo (e complexo) para a ecologia, o que culminou no conceito desenvolvido pelos pesquisadores Herbert Andrewartha e Charles Birch (1954), definindo *ecologia* como o estudo científico da distribuição e abundância dos organismos, conceito este que foi modificado e , mais tarde, substituído por uma forma mais prática sugerida por Charles J. Krebs 1972), que definiu a *ecologia* como sendo o estudo científico das *interações que determinam a distribuição e abundância dos organismos*. Assim, citando Begon e colaboradores (2007), na introdução do livro "*Ecologia, de indivíduos a ecossistemas"*, a definição proposta por Krebs em 1972 conseguiu localizar o cerne da ecologia: Onde os organismos ocorrem? Quantos ocorrem em um local e por quê apresentam este padrão de ocorrência? E, por não trazer escrita a palavra *ambiente*, deixou implícito que este, através dos seus fatores bióticos e abióticos (da interação deles), é capaz de determinar (gerando diferentes padrões) e responder às preguntas supracitadas. Desta forma, permite que os ecólogos façam estimativas do que acontecerá a uma espécie, uma população, uma comunidade e até mesmo a todo um ecossistema (Begon et al. 2007)

Os diferentes níveis da organização biológica definem uma espécie de hierarquia que podem ser chamados de *sistemas biológicos* (Pillar 2002). Essa hierarquia e esses sistemas podem ser estudados em diversas escalas de complexidade, desde genes e células - como exemplo de escalas menores - até ecossistemas e a própria biosfera em toda sua complexidade. Desta forma, podemos separar como principais objetos de estudo da ecologia, a ecologia de *indivíduos* (diferentes *organismos* dentro de uma mesma espécie), a ecologia de *populações* (interessada nas *espécies*), a ecologia de *comunidades* (cujo objeto de estudo são as diferentes *populações*) e suas relações como o ambiente e seus fatores *bióticos* e *abióticos*. Essa forma hierárquica de dividir os sistemas biológicos está não somente ligada à complexidade destes, mas também com o fluxo energético, a energia produzida em um nível, que passa para outro e assim sucessivamente, até que retorne ao nível mais basal da teia trófica.

Do ponto de vista da ecologia de comunidades, a meta principal é a busca do entendimento de como os agrupamentos de espécies, ou *assembleias* de espécies, se organizam no mundo natural, em um determinado local ou um determinado período de tempo, bem como de que maneira a interação entre as espécies, os indivíduos e o ambiente são influenciados e exercem influência nesta organização (McGill et al. 2006). Essa não é uma linha de pensamento nova, algumas obras como o livro “Geographical ecology: Patterns in the distribution of species ”, publicado em 1972 por Robert H. MacArthur, já haviam trabalhado a ideia de que o objetivo da ecologia de comunidades é encontrar os *padrões gerais* e, desde então, a ciência vem buscando identificar mecanismos que limitam ou moldam a distribuição das espécies ao longo dos ambientes e assim, melhorando o entendimento acerca dos diferentes padrões de diversidade, composição, abundância e até mesmo auxiliando na identificação de atributos que tenham contribuído (ou não) com o sucesso e com a manutenção das espécies nos ambientes (Werner e Glennemeier 1999).

De uma forma geral, mais moderna e ainda mais complexa, esses estudos têm levado em conta quatro principais padrões geradores de biodiversidade. São aqueles padrões representados pela *diferença de aptidão entre as espécies*, *pelas mudanças aleatórias nas abundâncias*, *pela geração de novas espécies* e *pelo movimento destas ao longo do ambiente* (Vellend 2010). O estudo destes padrões visa entender as chamadas “*assembly rules*” ou regras de montagem das comunidades, uma ideia já antiga proposta por Diamond (1975), de que existem conjuntos de fatores que restringem a formação ou a manutenção das comunidades, podendo serem definidas como os processos ecológicos (exercidos sobre as espécies) que determinam a estrutura das comunidades em um determinado local e tempo (Sobral e Cianciaruso 2012).

Estes padrões geradores de biodiversidade podem ser então avaliados através da *diversidade taxonômica*, utilizando as espécies ou unidades taxonômicas, da *diversidade* *funcional* - através da utilização de características de cada indivíduo de cada espécie - ou da *diversidade filogenética,* através das relações de parentesco. Ambas as abordagens se utilizam de atributos que mostram *efeito* sobre a *aptidão* dos indivíduos a permanecerem em seus ambientes, sendo estes fenotípicos, funcionais ou filogenéticos. Estas abordagens permitem entender processos ecológicos importantes como imigrações, extinções, e até mesmo eventos evolutivos que podem ter efeitos sobre a estrutura da comunidades e assim podem trazer importantes implicações nas políticas conservacionistas (Patterson 1990).

Nas últimas décadas, os avanços científicos e tecnológicos dentro e fora das ciências naturais têm permitido (ou ajudado) à ecologia acessar de forma mais precisa e mais rápida os fatores que regulam e influenciam o funcionamento dos ecossistemas e, de posse destas informações, torna-se possível lidar de forma mais acertada ou até mesmo facilita prever os problemas ambientais de forma mais rápida, em uma época de modificações climáticas, crises de biodiversidade e de dificuldades na implementação de formas sustentáveis de desenvolvimento. Em outras palavras, entender como a ação antrópica vem afetando (negativamente) a vida no planeta, bem como encontrar maneiras de recuperar ou salvaguardar o que ainda resta da saúde dos ecossistemas, uma tarefa que se torna mais e mais urgente.

A Mata Atlântica: histórico de modificações e degradação

A Mata Atlântica é a segunda maior floresta da região Neotropical (região que abrange o sul da América do Norte ao sul da América do Sul), sendo caracterizada por um conjunto de formações florestais e campestres (Ombrófila Densa, Ombrófila Mista, Estacional Semidecidual, Estacional Decidual e Ombrófila Aberta), que se estendia, originalmente desde o estado do Rio Grande do Norte (RN) até o Rio Grande do Sul (RS) de forma contínua, com faixas que podem chegar até 200km de largura da costa litorânea às regiões interioranas. Antes da exploração o bioma possuía uma área de aproximadamente 1,3 milhão de km^2, compreendendo então 17 estados da Federação, mas hoje reduzida a 232.939 fragmentos de floresta nativa com área maior de três hectares, totalizando menos de 11% do bioma original (Myers et al. 2000, “Fundação SOS Mata Atlântica” 2016) .

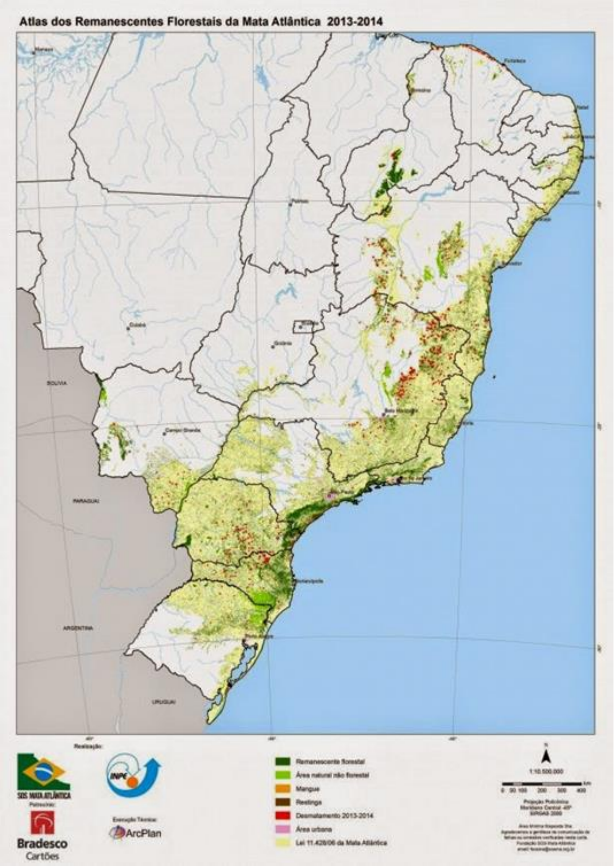

Figura 1. Mapa de abrangência da Mata Atlântica, com os remanescentes florestais e a zona original de domínio do bioma (Fonte: http://mapas.sosma.org.br/).

A história da devastação da Mata Atlântica está intimamente ligada ao desenvolvimento socioeconômico do Brasil. O ciclo de sobre-exploração teve início com a firmação do primeiro contrato de exportação de Pau-Brasil (*Paubrasilia echinata*), já em 1502 (Florestas 2016) Instituto Brasileiro de Florestas. Daí para frente, a floresta ainda passou por outros ciclos, como o do ouro, o da cana-de-açúcar e do café ao final do século XIX e início do século XX. Atualmente a Mata Atlântica enfrenta a ampliação das áreas urbanas (mais de 3.000 municípios brasileiros tem seus territórios dentro do bioma) e o avanço das fronteiras agrícolas devido especialmente às pastagens para gado e monoculturas da soja, milho e trigo. Estas atividades são e foram geralmente beneficiadas pela alta fertilidade dos solos, pelas políticas administrativas ineficazes (ou corruptas) e pela severa restrição financeira originada pela má administração dos recursos públicos (Murphy e Lugo 1986, Prado e Gibbs 1993, Peres e Terborgh 1995).

Além da ampliação da área urbana/agrícola, existem ainda a extração ilegal de madeira, o comércio ilegal/ tráfico de plantas e animais, a pesca predatória e a caça, fatores que nos dias de hoje seguem deplecionando a vida silvestre no bioma (Laurance 1999, Cullen et al. 2000, Tabarelli et al. 2004), gerando prejuízos incalculáveis, modificando os padrões espaciais da paisagem e acelerando processos como a perda de habitat (Dean 1996, Maes 2004). Desta forma, os poucos remanescentes relativamente protegidos dos ecossistemas da Mata Atlântica encontram-se hoje isolados em pequenas unidades de conservação, o que tem gerado crescentes problemas tanto nas esferas ambientais quanto nas sociais (Brown et al. 1992, Stagl et al. 2014), graças aos conflitos de interesse entre moradores, agricultores, políticos e ambientalistas.

Parque Estadual do Turvo: o maior remanescente de floresta estacional protegido no Rio Grande do Sul

Dado seu histórico de devastação, os ecossistemas florestais relacionados à Mata Atlântica *lato sensu* estão entre os que sofrem maior pressão antrópica. No Brasil, 70% da população encontra-se vivendo dentro dos domínios deste bioma, gerando uma necessidade urgente de proteção de seus remanescentes. Desta forma, a implantação de Unidades de Conservação é amplamente reconhecida como uma estratégia para a conservação *in situ* da biodiversidade, além de proteger o meio físico, preservar o patrimônio histórico-cultural associado a ambientes naturais, bem como os serviços ambientais (McNeely e Mackinnon 1989).

A ocupação expressiva da área em que hoje se encontra o Parque Estadual do Turvo (PET), no Rio Grande do Sul, teve início em meados da década de 1930, sob orientação de Getúlio Vargas, quando começaram a ser traçados planos para ampliar o processo de colonização do sul do Brasil. Não diferente do que ocorreu em outras partes do bioma, a colonização da área foi facilitada pela alta fertilidade dos solos, em sua maioria recém expostos graças à extração de madeira. Este tipo de atividade abriu clareiras nas florestas, gerando renda a partir da venda de madeira e, assim, possibilitando o estabelecimento de culturas secundárias para subsistência das famílias em meados de 1950. Posteriormente, quando já consolidadas as propriedades rurais de subsistência, estabeleceram-se as primeiras criações de suínos por volta de 1960 e, em seguida, a expansão das monoculturas em meados de 1970 até o presente.

O Parque Estadual do Turvo foi criado através do Decreto Estadual nº 2.312, de 11 de março de 1947 e foi uma das primeiras unidades de conservação instituídas no Rio Grande do Sul. Posteriormente, no ano de 1954, a Lei nº 2.440, de 02 de outubro determinou que todas as matas de domínio do estado e com mais de 250 ha fossem transformadas em Parques Estaduais. Desta forma, o PET tornou-se a maior área protegida legalmente (Unidade de Proteção Integral) do estado do Rio Grande do Sul e o último grande remanescente de Mata Atlântica do sul do Brasil, bem como um dos últimos redutos representativos da vegetação original da mata pluvial do Alto Uruguai (Brack et al. 1985).

O Parque Estadual do Turvo está situado no extremo noroeste do Estado do Rio Grande do Sul, entre as coordenadas 27°07 a 27°16 de latitude Sul e 53°48 a 54°04 de longitude Oeste, no município de Derrubadas, junto ao Rio Uruguai, fazendo divisa com o estado de Santa Catarina e a província argentina de Misiones (SEMA 2005). O Parque conta com uma área de 17.491,4 ha, o que representa quase 50% da área total do município de Derrubadas (Figura 2). A temperatura média anual é de 19,1 °C e a precipitação pluviométrica é de 1.800 mm. O relevo do município tem características predominantemente onduladas e acidentadas, com aproximadamente 5% da área plana (SEMA 2005, Rosa et al. 2013). O IBGE (1986) inclui toda a mata do alto Uruguai na Região Fitoecológica da Floresta Estacional Decidual que, originalmente, estendia-se em direção sul até a região das Missões e até a bacia do Rio Ibirapuitã, nas proximidades da cidade de Alegrete-RS. Outros estudos caracterizam a vegetação do PET como (ver Iop et al. 2011). Independentemente da aparente falta de consenso, as florestas sazonais são conhecidas como as mais ameaçadas do mundo dado suas características climáticas e de solo (Jansen 1997), favoráveis à agricultura e pastejo, como dito anteriormente.

Figura 2. Localização do Parque Estadual do Turvo no município de Derrubadas- RS, extremo noroeste do estado do Rio Grande do Sul, fazendo fronteiras ao norte com o estado de Santa Catarina, e a oeste com a Província de Misiones (Argentina).

Anfíbios: Quem são, onde estão e quantos são?

Os primeiros anfíbios começaram a surgir ao final da segunda metade do período Devoniano e no início do período Carbonífero, isto é, há cerca de 350 milhões de anos atrás, uma era onde grande parte da superfície terrestre estava tornando-se coberta por áreas pantanosas, um período de cheias e secas intermitentes, o que permitiu (ou exigiu?) que muitos dos grupos de vertebrados da época passassem por severas adaptações, tanto morfológicas quanto comportamentais. Os primeiros ancestrais dos anfíbios modernos evoluíram a partir de peixes Osteolepiformes (Figura 3) que desenvolveram as chamadas “nadadeiras lobadas”, estruturas locomotoras que deram origem aos membros dianteiros e traseiros dos primeiros tetrápodes, e assim, são comumente considerados uma transição entre os peixes e os amniotas (lagartos, serpentes, crocodilianos, quelônios, aves e mamíferos). A presença de quatro membros locomotores (daí o nome tetrápode) mais robustos e articulados (um par peitoral e um par pélvico) ajudou a aprimorar a capacidade se deslocar e de sustentar o peso do corpo em ambientes rasos bem como desenvolvimento de pulmões eficientes, adaptações que foram cruciais para a diversificação do grupo nos ambientes terrestres. Hoje existem aproximadamente 7.000 espécies de anfíbios ao redor do mundo. Desta forma, podemos dizer que os anfíbios lideraram a colonização do ambiente terrestre pelos vertebrados em uma época em que o ambiente fora d’água era dominado por invertebrados (artrópodes em sua maioria).

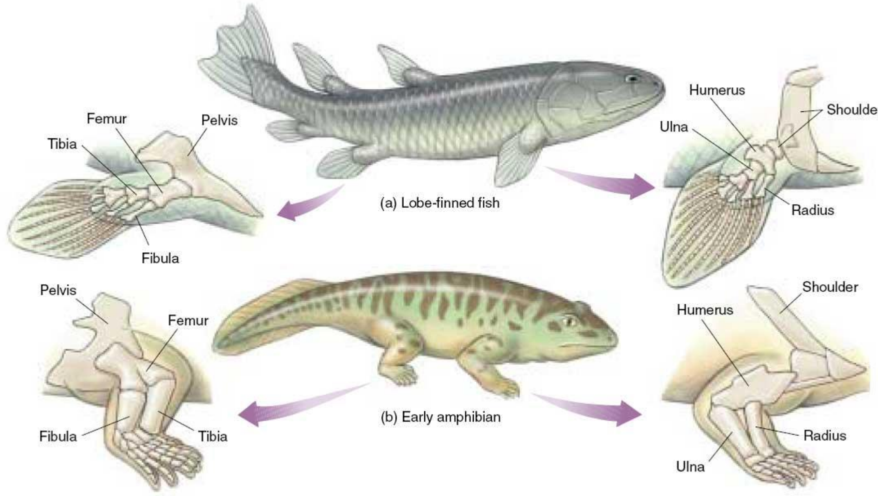

Figura 3. Os primeiros tetrápodes (Fonte: “Biology”, P. Raven e G. Johnson, 6a ed., Mcgraw-Hill)

A classe Amphibia, atualmente é dividida em três ordens, Gymnophiona (cecílias), Urodela (salamandras e tritões) e Anura (sapos, rãs e pererecas). Apesar do sucesso destes grupos em conquistar os ambientes terrestres os anfíbios atuais, assim como seus ancestrais, permaneceram dependentes da água disponível no ambiente. O fato dos anfíbios terem seus ovos desprovidos de casca (estrutura porosa que protege o embrião e retarda a desidratação fora d'água, presente m serpentes, lagartos aves e alguns mamíferos) e das membranas extraembrionárias (Âmnio, Córion e Alantoide, responsáveis pela proteção mecânica e trocas gasosas entre o embrião e o ar, respectivamente), fez com que este grupo de vertebrados tetrápodes se mantivesse utilizando a mesma estratégia reprodutiva que seus parentes, depositando os ovos dentro da água e dando assim origem a girinos fisiologicamente semelhantes a peixes. No entanto, olhando mais atentamente, podemos ver que as semelhanças neste estágio vão pouco além de hábitos aquáticos, presença de uma linha lateral e respiração branquial. Desde a saída do ovo, o recém-nascido já apresenta características próprias dos anfíbios, como pele extremamente úmida e permeável (relacionada à respiração cutânea), brânquias externas, coração com três câmaras, bem como ausência de escamas, pelos ou penas comuns a todos os demais grupos de vertebrados.

A capacidade de explorar ambientes aquáticos e terrestres exigiu dos anfíbios, ao longo de seu processo evolutivo, a diversificação não só de caracteres morfológicos e comportamentais. A colonização de novos ambientes fez com que os anfíbios tivessem de diversificar também seus modos reprodutivos, a fim de lidar com as novas exigências impostas pelos diferentes ambientes. Desta forma, agora nem todos os anfíbios irão depender totalmente da água para completar seus ciclos de vida. Muitas espécies desenvolveram modos reprodutivos com grau variado de independência da água, como por exemplo, os ovos depositados em folhas por espécies dos gêneros *Phyllomedusa* e *Pithecopus,* ou mesmo ovos e recém eclodidos desenvolvendo-se em pedras úmidas ou no solo da floresta, como em várias espécies dos gêneros *Cycloramphus* e *Thoropa* (Haddad e Prado 2005).

Desta forma, os anfíbios tornaram-se o grupo com maior diversidade de modos reprodutivos, ficando atrás apenas dos peixes. Esta alta diversidade de atributos morfológicos, comportamentais e reprodutivos é um dos motivos pelos quais peixes e anfíbios são dois dos grupos de vertebrados mais bem distribuídos no planeta, aos anfíbios excetua-se apenas as regiões polares, as quais estes não conseguem se estabelecer graças a seus requisitos fisiológicos.

Problemas relacionados à conservação dos anfíbios anuros

A ordem Anura é representada por aproximadamente 6.600 espécies de anfíbios formalmente descritas ao redor do mundo (Frost 2016). Estes são, em sua maioria, bem distribuídos em todos os continentes, com exceção da Antártida onde não ocorrem.

Segundo a Sociedade Brasileira de Herpetologia, o Brasil possui 1.039 espécies de anuros formalmente registradas (Segalla et al. 2016), o que torna o Brasil o recordista mundial em riqueza de espécies. Destas, aproximadamente 500 ocorrem na Mata Atlântica (Haddad et al. 2013) e aproximadamente 90 espécies são endêmicas do bioma. No entanto, a Mata Atlântica não é recordista apenas em número de espécies, aproximadamente 70% (n=27) dos modos reprodutivos registrados para o mundo todo ocorrem na Mata Atlântica (Pombal e Haddad 2007). Sendo assim, pode-se inferir que aproximadamente 10% de todas estas espécies de anuros já conhecidas pela ciência podem ser encontradas no bioma Mata Atlântica. Segundo Myers (1988) e Myers ecolaboradores (2000) esta concentração excepcional de espécies somada ao número excepcional de endemismos tornam a Mata Atlântica uma zona de relevância ecológica e assim, um dos 34 *hotspots* na agenda para conservação da biodiversidade, bem como um dos 25 ecossistemas mais ameaçados no planeta, segundo a *Conservation International* (CI).

De acordo com a União Internacional para a Conservação da Natureza (IUCN), os anfíbios são o grupo mais ameaçado dentre os vertebrados, considerados mais ameaçados que as aves e os mamíferos (Blaustein et al. 2011). Dadas as características fisiológicas e as limitações de distribuição, os anfíbios estão enfrentando taxas de extinção 211 vezes maiores que as taxas esperadas (Blaustein et al. 2011). Dentre as ameaças sofridas pelos anuros, a fragmentação e a perda de habitat são alguns dos problemas mais recorrentes e mais facilmente detectados (Bowne e Bowers 2004). Os eventos de extinção, cada vez mais comuns, são geralmente associados a diversos outros fatores, por vezes negligenciados. Dentre eles, estão o aumento na incidência de radiação UV nas áreas sob efeito de fragmentação e desflorestamento (Anzalone et al. 1998, Blaustein et al. 1998, Lipinski et al. 2016), a introdução de patógenos contaminantes (Carnaval et al. 2006), a introdução de espécies exóticas e invasoras (Fellers e Drost 1993, Kiesecker e Blaustein 1997), a contaminação por pesticidas (Josende et al. 2015) e por fim os declínios populacionais "enigmáticos", em áreas isoladas ou protegidas, atribuídos às flutuações naturais destas populações (Pechmann e Wilbur 1994).

Desta forma, as constantes modificações ambientais fazem com que muitas das espécies sob tais pressões ambientais já tenham, tanto em nível local quanto global (Bergman e Landin 2002, Maes 2004), perdido suas áreas de distribuição natural, tenham sofrido declínios populacionais ou tenham sido extintas antes mesmo de serem estudadas pela ciência (Blaustein e Wake 1990). Essa situação torna-se ainda mais alarmante considerando que diversos estudos (ver Stuart et al. 2004; McCallum 2007; Hayes et al. 2010; Blaustein et al. 2011) demonstram que os eventos de declínio/extinção de anfíbios são mais severos e mais bem distribuídos globalmente do que o esperado por variações demográficas normais (Pounds e Crump 1994, Pounds et al. 1997, 2006, Stuart et al. 2004). Assim, conhecer os mecanismos responsáveis pela exclusão ou manutenção das espécies nos ambientes naturais e antropizados é o meio mais preciso para elaborar medidas que visem preservar, tanto espécies quanto ecossistemas.

Apresentação

A presente Tese foi dividida em quatro capítulos e estruturada de acordo com as normas da 'Estrutura e apresentação de monografias, dissertações e teses: MDT', da Universidade Federal de Santa Maria (MDT, 2012).

Capítulo I: *"An UV-sensitive anuran species as an indicator of environmental quality of the Southern Atlantic Rainforest"* - Journal of Photochemistry & Photobiology, B: Biology 165 (2016) 174–181.

Neste capítulo avaliamos os efeitos da radiação UV sobre o desenvolvimento embrionário, taxas de mortalidade e desenvolvimento de malformações em girinos de *Hypsiboas curupi* uma espécie endêmica e ameaçada de extinção.

Capítulo II: *"Structure and distributional patterns of anuran assemblages along a forest-agricultural gradient" – Community Ecology (submetido)*

Neste capítulo avaliamos de que forma as comunidades presentes no interior e no exterior da unidade de conservação se comportam, ao longo de um gradiente ambiental de distância em relação à borda.

Capítulo III: *"Changes on anuran tadpole functional diversity along an environmental gradient at the southernmost Atlantic Rainforest remnant"*

Neste capítulo buscamos entender de que forma os estágios larvais dos anuros respondem às modificações da paisagem e dos corpos d'agua utilizando algumas medidas de diversidade funcional.

Capítulo IV*: "Enhanced phylogenetic diversity: a result of species loss in an agricultural environment"*

Neste capítulo avaliamos aspectos de diversidade funcional, taxonômica e filogenética das comunidades de anuros presentes ao longo do já mencionado gradiente ambiental e exploramos a relação deste, com o aumento ou diminuição nos padrões de diversidade.

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CAPÍTULO I

An UV-sensitive anuran species as an indicator of environmental quality of the Southern Atlantic Rainforest

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An UV-sensitive anuran species as an indicator of environmental quality of the Southern Atlantic Rainforest

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ABSTRACT

The Southern Atlantic rainforest is continuously suffering from wood extraction activity, which results in the increase of clearings within the forest. Although the direct impacts of deforestation on landscape are already well described, there is an absence of studies focused on the evaluation of its indirect effects, such as the increase of solar UV radiation levels inside forest environment and its consequences for forest specialist anuran species. The results presented in this work clearly show that the threatened tree frog species *Hypsiboas curupi* presents severe traits of sensitivity to UV wavelengths of sunlight, making it a vulnerable species to this environmental stressor, as well as a biological indicator of the quality of forest canopy coverage. In addition, the measurement of solar UVB and UVA radiation incidence upon *H. curupi* breeding site and the analyses of a 20-year dataset of satellite images regarding the management of canopy coverage indicate that the photoprotection provided by trees of the Southern Atlantic rainforest is critical for the conservation of this forest specialist anuran species. Therefore, this work demonstrates that the deforestation process enhances the exposure of *H. curupi* embryos to solar UVB and UVA radiation, negatively affecting their embryonic development, inducing mortality and population decline.

Keywords: Deforestation; UV radiation; Amphibian decline; Mortality; Malformation.

1. Introduction

Since the first reports of the global phenomenon of amphibian decline, many species have become extinct or have faced reduction in their natural distribution ranges [1,2]. Among several factors related to this process, forest canopy is historically under severe anthropogenic pressure [3,4]. Canopy removal has been shown to have long-term negative effects on amphibian abundance, with some populations taking two to seven decades post-harvesting to reach pre-disturbance levels [5–8]. In addition, it has been also demonstrated that forest specialist amphibian species decline in abundance after forest disturbance while most habitat generalist species are not affected by canopy removal, thereby indicating a shift in the relative abundance towards habitat generalists [9].

Similar observations were also obtained at the Brazilian Atlantic Rainforest. This biome has lost 88% of its original area [10], and it is suggested that approximately 30% of endemic anuran species are facing decline due the suppression/change of the native forest by anthropogenic activities [11,12]. More specifically, the southernmost region of this forest has been facing a gradual deforestation since 1910, which resulted in the increase of open areas within the forest [13]. Consequently, in 1947 the government of the Rio Grande do Sul state created the Turvo State Park (TSP), which is a conservation unit that covers more than 17,000 hectares of the Southern Atlantic rainforest [14]. However, the management plan of the park was incipient until the beginning of the year 2000, which prompted the state government to apply new conservation measures that began to be strictly implemented in this area [14].

An important consequence of canopy removal in Brazilian territory is the incidence of very high levels of solar UV radiation inside this environment. Indeed, UVB radiation has a major role in the occurrence of amphibian population decline due to its genotoxic impact during the embryonic and larval stages [15–18]. On the other hand, amphibian photolyases play the main role in the repair of DNA damage induced by sunlight, such as the cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine-pyrimidone photoproducts (6-4PPs) [19–22]. However, it has been recently shown that tree frog tadpoles present low efficacy in repairing both CPDs and 6-4PPs induced by UVB and UVA radiation, which resulted in the generation of serious biological effects on the development of these organisms (i.e., changes on body mass and total length, as well as induction of malformation after metamorphosis and mortality) [23]. Furthermore, it is predicted that anuran species whose eggs are naturally highly exposed to sunlight have higher enzymatic activity than species whose eggs are naturally protected [24].

Despite the wide knowledge in the field regarding the effects of forest harvesting on the landscape, there is an absence of studies showing its impact on the increase of solar UV radiation incidence inside forest environment, as well as its biological consequences to forest specialist anuran species. In the present work, we measured the influence of deforestation on the solar UVB (280-315 nm) and UVA (315-400 nm) radiation incidence upon breeding sites of the tree frog species *Hypsiboas curupi*. This species was chosen as a model because it is currently restricted to well conserved (highly forested) portions of streams inside the Southern Atlantic rainforest [25]. Despite *H. curupi* is listed as least concern at IUCN list, both national and state lists consider it as an ‘endangered’ species because of its very fragmented distribution on portions of Southern Atlantic rainforest with anthropogenic pressure, which are generally located outside conservation units [26,27]. Therefore, this species was recently included as a target-species on the National Action Plan for the Conservation of Southern Brazil's Herpetofauna, thus requiring more scientific studies focused on its biology and ecology [27].

The detrimental effects of solar-simulated UV doses were addressed in terms of hatching success, developmental rates, mortality, and malformation induction in embryos and newly hatched tadpoles. Additionally, the management of the canopy coverage over the TSP’s area was also evaluated through the analyses of 20 years of satellite images. By comparing the biological data with UV light incidence and satellite images, we demonstrate the importance of conservation politics focused on maintaining this biome in order to avoid the severe biological effects of sunlight upon UV-sensitive populations of this endangered amphibian species.

2. Material and methods

2.1. Solar UVB and UVA radiation measurement

Daily incidence of UVB and UVA radiation was measured during the entire daylight period (6:30 am to 7:00 pm, local time) at the breeding site of the threatened tree frog species *Hypsiboas curupi* (on the edge of a stream inside of a highly conserved forested area), as well as in a converted area (deforested area) of the same stream, both inside the Turvo State Park (TSP) domain, Rio Grande do Sul, Brazil (27°07’ – 27°16’S, 53°48’ – 54°04’W), which is the largest remaining fragment of Mesophytic Semideciduous Forest of the state [14]. These measurements were performed at one clear sky summer day (at February, 2014 between day 11 and day 26) in each area through the use of a portable radiometer (EKO UV Monitor MS-211-1, Japan).

2.2. Animal collection and maintenance

Despite the fact that *H. curupi* is listed as an ‘endangered’ species on both national and state lists, we also decided to choose this species to link the effects of solar UV radiation with deforestation process because of the fact that the females deposit the egg masses floating on the water surface (above the water surface) at shallow streams, usually attached to rocks or floating branches (this is called Reproductive mode 2 [28–30]). Four freshly laid egg masses of *H. curupi* were collected in the early morning. The egg masses were packed in plastic sacs half filled with air and water from stream to avoid physical shocks during the transportation to the laboratory. Then, the embryos were counted and, immediately, the viable individuals were randomly separated and placed individually into 2.0 ml microtubes. The total amount of microtubes containing one viable embryo formed 9 groups with 90 embryos each in order to perform three independent exposures of 30 individuals to each UVB and UVA dose applied in this work, as well as to compose the non-irradiated control groups.

2.3. UVB and UVA irradiation treatments

Each replicate containing 30 embryos were placed together in Petri dishes at a distance about 30 cm from the light sources, and irradiated independently with increasing UVB (0.5, 1.0, 2.0, and 4.0 kJ/m^2) and UVA (25, 50, 100, and 200 kJ/m^2) doses. In parallel to UV irradiation, each replicate of 30 embryos of the non-irradiated control were also placed in a Petri dish filled with dechlorinated water (19°±1°C) and kept under the ambient light until the end of UV treatments (for 36 min). UVB exposures were performed with a Vilber Lourmat T15 M 15 W lamp (Vilber Lourmat, France) filtered with a polycarbonate sheet to block UVC wavelengths. For UVA, tadpoles were irradiated using an Osram Ultramed FDA KY10s 1000 W lamp filtered with Schott BG39 glass filter, 3 mm thickness (Schott Glass, Germany). During irradiation, the water temperature measured under both UV-filters was 20°C (±1°C), and the amount of UVC contamination for the UVB lamp as well as UVC and UVB contamination for the UVA lamp was below the detection limit. The highest UV doses applied in this work correspond to 10% of daily solar UVB and UVA doses measured at the converted area, and the exposure times to achieve these energies were 17 min for the UVB and 36 min for the UVA lamps.

2.4. Effects induced by UV doses on hatching, survival, and morphology of H. curupi

After UV treatments, all embryos (including controls) were again separated and placed individually in 2.0 ml microtubes filled with dechlorinated water (19°±1°C) until the time of hatching. The eggs were analyzed daily to evaluate the success of hatching among non-irradiated controls and UV-exposed embryos. After the period of 96 h, the non-hatched individuals were considered dead. This period was defined *a posteriori* since all non-irradiated control embryos hatched within 96h and no UV-treated embryos could hatch after this period. After hatching, the mass of each tadpole was measured with an analytical balance (Radwag WTB 2000, Poland), and all tadpoles from the non-irradiated control group and from each UV treatment were placed together in their respective tank (according to the treatment) containing 1 liter of dechlorinated water (19°±1°C). The water volume was replaced every day to maintain the water quality and tadpoles were fed with boiled spinach *ad libitum.* The newly hatched tadpoles were also monitored for an additional period of ten days (240 h) with the purpose of obtaining the mortality rates (number of dead tadpoles during this period), and the occurrence of malformations due to UVB or UVA exposures (Nova Optical Systems stereomicroscope with 40X magnification, Brazil). To avoid human contact with the eggs and tadpoles, we used an adapted siphon to split and move the eggs to the microtubes, as well as to move the newly hatched tadpoles from the microtubes to the plastic tanks.

2.5. Analyses of a 20 years dataset of satellite images for the evaluation of forest canopy over the TSP's area

Satellite images were used to investigate the changes on forest canopy during the period of 1990 to 2010 over the TSP's area. These images were georeferenced for a posterior classification to separate, in distinct colors, the deforested areas (red spots) from the preserved areas (green), in order to calculate the total size of both areas in each decade separately (1990-2000; 2000-2010). The result is presented as the percentage of preserved area in each decade. All images and dataset were obtained from the LANDSAT 5 satellite (TM sensor) from the National Institute of Space Research (INPE, Brazil). The analyses of these images were performed through the use of the ArcGis 10.1 software (Esri, USA).

2.6. Statistical analysis

For the experiments of success of hatching we used the number of hatched tadpoles (expressed as the average number of hatched tadpole of three independent experiments) as the response variable, and the exposures to UVB or UVA doses, as well as the time of each analysis (24, 48, 72, and 96 h) as predictors.

For the experiments of embryonic mortality rates we used the number of dead embryos (expressed as the average percentage of dead embryos of three independent experiments) as the response variable, and the exposures to the UVB or UVA doses as predictors.

For the experiments to evaluate the changes in the mass of newly hatched tadpoles we used the mass values (expressed as the average of mass (g) of three independent experiments) as the response variable, and the exposures to the UVB or UVA doses as predictors.

For the tadpoles' mortality rates we used the number of dead tadpoles (expressed as the average percentage of dead tadpoles of three independent experiments) as the response variable, and the exposures to the UVB or UVA doses as predictors.

All the results produced in the experiments described above were analyzed and mutually compared by ANOVA with repeated measures, followed by Post Hoc Tukey's tests ($p < 0.05$) using the software GraphPad Prism 6.0 (GraphPad Software, USA).

3. Results

The daily profiles of solar UVA and UVB radiation incidence in both preserved and deforested areas are presented in Figure 1.

The UV doses measured in the deforested area were 42.3 kJ/m^2 of UVB and 1,786.9 kJ/m^2 of UVA, while in the preserved area it dramatically decreased to 1.54 kJ/m^2 of UVB and 124.1 kJ/m^2 of UVA.

The biological effects of both UVB and UVA radiation on *H. curupi* embryos was first evaluated in terms of success of hatching. This result is presented in Figure 2 and the statistics in Table 1.

Surprisingly, all UV doses applied in this work delayed the time of hatching in comparison to the non-irradiated control group. Interestingly, the lower UVA dose (25 kJ/m^2) induced an expressive delay on hatching time; the tadpoles started to hatch only 72 h after this treatment. On the other hand, the treatments with the UVB doses of 1 and 2 kJ/m^2, and with the UVA doses of 100 and 200 kJ/m^2 resulted in a large accumulation of hatched tadpoles in the period of 48 h (82% and 89% for 1 and 2 kJ/m^2 of UVB, respectively; 92% and 68% for 100 and 200 kJ/m^2 of UVA, respectively).

In addition, the impact of both UVB and UVA exposures was also evaluated in terms of embryonic mortality, as presented in Figure 3.

Curiously, only the higher UVB dose (4 kJ/m^2) resulted in a severe and statistically significant embryonic mortality ($F_{(4.00,\ 8.00)} = 54.52$; $p < 0.0001$; DF = 8).

After hatching, the mass of each tadpole was first evaluated, and this result is presented in Figure 4.

All UVB doses induced a significant loss of body weight in comparison to the non-irradiated control samples ($F_{(4,\ 146)} = 9.705$; $p < 0.0001$; DF = 146). Interestingly, after UVA treatments, only the lower UVA dose (25 kJ/m^2) induced a statistically significant decreased on tadpoles' mass ($F_{(4,\ 275)} = 5.584$; $p < 0.0001$; DF = 275).

Additionally, two types of malformation could be observed in the hatched tadpoles from the UVB treatments. Representative examples of tadpoles with these malformations are presented in Figure 5.

Following these analyses, tadpoles were also monitored for a period of 240 h in order to evaluate the mortality induced by both UVB and UVA treatments. This result is presented in Figure 6 and the statistics in Table 2.

Clearly, all UVB doses induced detrimental effects on *H. curupi* embryos that resulted in high mortality levels of the newly hatched tadpoles. Curiously, the lower UVA doses (25 and 50 kJ/m^2) induced a higher mortality than the higher UVA doses (100 and 200 kJ/m^2).

Finally, considering the high sensitivity of this forest specialist tree frog species to the solar UV radiation and the intense timber activity that historically occurred within the TSP conservation unit, a dataset containing two decades (1990 to 2000, and 2000 to 2010) of satellite images regarding the forest canopy over this area was analyzed, and the result is presented in Figure 7.

This analysis shows that the wood extraction activities dramatically decreased during the last decade (2000-2010), allowing an expressively increase in the proportion of the preserved forested area over the domain of this conservation unit.

4. Discussion

It is well known that forest canopy is an important determinant for conservation of amphibian diversity [3,31]. Despite habitat generalist species are less influenced by the forest harvesting, strong negative responses of forest specialist amphibian species to clearcutting (i.e., avoidance, low abundance) have been reported [9]. This occurs because forest disturbance creates landscapes that tend to favor generalist species, while negatively affecting specialist species [32].

Despite the increasing knowledge regarding the general consequences of forest harvesting on the richness and abundance of forest restricted amphibian populations, little is known about the protection efficacy of canopy against the harmful effects of solar UV radiation to these organisms. The majority of published studies consider the canopy only as an environmental variable that promotes shelter against predation or other biological interactions [33–35]. Here we show that the canopy of Southern Atlantic rainforest provides a robust barrier against the incidence of very high doses of solar UVB and UVA radiation (Fig. 1). The protection efficacy of forest canopy is even more evident during the peak of sunlight incidence (period from 11:30 a.m. to 2:00 p.m.), reaching values of 99% for UVB and 96% for UVA wavelengths.

In addition, this work demonstrate that the threatened tree frog species *Hypsiboas curupi* is hypersensitive to the UV component of sunlight, since very low UVB and UVA doses induce detrimental effects on the development (Fig. 2) and survival (Fig. 3) of embryos, as well as on the morphology (Fig. 4 and Fig. 5) and survival (Fig. 6) of newly hatched tadpoles. The ecological implications of these results extend the individual responses obtained with *H. curupi*. The enhanced mortality of embryos and newly hatched tadpoles can affect the entire population dynamics, due the reduction of the recruitment of larval and juvenile stages [36,37]. Consequently, this can result in losses of important components of diversity and ecological services, which can help to explain the enigmatic decline of amphibian species [38–41]. Furthermore, one published study with other amphibian species that possess similar reproductive mode of *H. curupi* (*Hyla cadaverina*, *Hyla regilla* and *Taricha torosa*), but does not occur alongside with *H. curupi* in nature, also presented similar results [42]. In fact, amphibian embryos are directly subjected to the environmental stress induced by sunlight because they cannot avoid UV radiation as some larvae or adult animals do [18,43]. Thus, this makes the forest canopy the most important protection factor against sunlight exposure during the embryonic life stage of forest specialist amphibian species [44].

It is already known that the damage induced by environmental stressors can trigger different developmental responses by amphibian embryos [45–49]. The hatching time is regulated by physiological mechanisms, and it could be delayed or accelerated in response to external stimuli [50]. Thus, our results also indicate that solar UVA and UVB radiation can affect the time of embryonic development of *H. curupi* (Fig. 2). Therefore, in order to reduce UV-induced embryonic mortality, the hatching time is changed, probably as an attempt to produce better-developed hatchlings with better feeding potential and ability to cope with the size-and-stage dependence [45,50–52]. In fact, despite all UV doses applied in this work delayed the time of hatching in comparison to the non-irradiated embryos (Fig. 2), only the higher UVB dose (4 kJ/m^2) induced a significant mortality rate (Fig. 3).

The impact of UV radiation on the mass of hatched tadpoles observed in this work (Fig. 4) can also have important ecological consequences on population dynamics, as previously described [51,53–55]. The deficiency on gaining weight presented by the UV-irradiated tadpoles is probably related to an excessive consumption of energy to repair the UV-induced DNA damage, since it is manifested as an energy demand process that can induce changes in cell composition, decreasing growth and survival rates [56,57]. In addition, the UVB-induced malformations on the hatched tadpoles observed in this work (Fig. 5) are similar to those found in earlier studies [18,43,58,59]. Our result clearly show the formation of a kinky tail that induces a distortion on the axial skeleton and promotes an erratic swimming, as well as a tegument hyperplasia (edema), which inflates the abdominal region and keeps the tadpole floating, making swimming impossible.

The vulnerability traits of a species are determined by at least two factors: one is related to intrinsic factors (i.e., sensitivity to enhanced UV levels inside the forest), while the other is related to extrinsic factors (i.e., exposure to an environmental factor not governed by the individuals, such as solar UV radiation) [60,61]. Our results clearly indicate that this tree frog species is extremely sensitive to solar UV radiation, since the exposure of embryos to very low UVB and UVA doses can also induce high mortality levels on hatched tadpoles (Fig. 6). Therefore, here we hypothesize that the high vulnerability of *H. curupi* to solar UV radiation is related to a low activity of the DNA repair enzymes photolyases. Since *H. curupi* inhabits streams with a high percentage of canopy cover, photolyases activity could not be necessary because this species is naturally well protected from sunlight. This probably can explain why the lower UVA doses used in this work induced a higher delay in the embryonic development and mortality of hatched tadpoles. On the other hand, during the exposures to the higher UVA doses these enzymes could be activated due to the longer exposure period under this light source, thus allowing the repair of the majority of UVA-induced DNA damage. However, further studies are still necessary to confirm this hypothesis.

Furthermore, considering that forest canopy is the most important protection against the sunlight exposure of *H. curupi* embryos, as well as the intense timber activity of Southern Atlantic rainforest since the decade of 1910, our results clearly show that the implementation of more rigorous preservationist procedures in the beginning of the last decade (2000) has been working indeed, reducing the wood extraction activity and increasing canopy coverage inside TSP's area (Fig. 7). Consequently, despite the reported decline of this amphibian species in the Southern Brazilian territory [26,27], *H. curupi* still presents high abundance inside the TSP conservation unit domain.

This is the first work showing that the threatened tree frog species *Hypsiboas curupi* presents high sensitivity to the UV wavelengths of sunlight, making it a vulnerable species to this environmental stressor. The deleterious effects induced by the UVB and UVA doses applied in this work upon *H. curupi* are of paramount importance for many other declining anuran species that live in Southern Atlantic rainforest. Moreover, this work indicates that timber activity in this biome can enhance the solar UV radiation levels upon breeding sites, increasing the risks of decline of forest specialist amphibian populations.

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Tables

Treatment	DF	F	CI (95%)	p
Control (24h) vs. UVB 05 kJ/m^2 (24h)	8	$F_{(4.000, 8.000)} = 39.17$	3.815 to 8.185	< 0.0001
Control (24h) vs. UVB 1 kJ/m^2 (24h)	8	$F_{(4.000, 8.000)} = 39.17$	4.148 to 8.518	< 0.0001
Control (24h) vs. UVB 2 kJ/m^2 (24h)	8	$F_{(4.000, 8.000)} = 39.17$	4.148 to 8.518	< 0.0001
Control (24h) vs. UVB 4 kJ/m^2 (24h)	8	$F_{(4.000, 8.000)} = 39.17$	4.148 to 8.518	< 0.0001
Control (48h) vs. UVB 4 kJ/m^2 (48h)	8	$F_{(4.000, 8.000)} = 11.92$	1.766 to 22.90	0.0231
Control (72h) vs. UVB 4 kJ/m^2 (72h)	8	$F_{(4.000, 8.000)} = 24.84$	8.347 to 24.99	0.0008
Control (96h) vs. UVB 4 kJ/m^2 (96h)	8	$F_{(4.000, 8.000)} = 40.62$	16.73 to 31.27	< 0.0001
Control (24h) vs. UVA 25 kJ/m^2 (24h)	8	$F_{(4.000, 8.000)} = 10.52$	2.620 to 10.05	0.0024
Control (24h) vs. UVA 50 kJ/m^2 (24h)	8	$F_{(4.000, 8.000)} = 10.52$	1.620 to 9.047	0.0071
Control (24h) vs. UVA 100 kJ/m^2 (24h)	8	$F_{(4.000, 8.000)} = 10.52$	0.9529 to 8.380	0.0154
Control (24h) vs. UVA 200 kJ/m^2 (24h)	8	$F_{(4.000, 8.000)} = 10.52$	1.286 to 8.714	0.0104
Control (48h) vs. UVA 25 kJ/m^2 (48h)	8	$F_{(4.000, 8.000)} = 42.99$	8.916 to 24.42	0.0005
Control (48h) vs. UVA 100 kJ/m^2 (48h)	8	$F_{(4.000, 8.000)} = 42.99$	-19.75 to -4.249	0.0045
Control (72h) vs. UVA 100 kJ/m^2 (72h)	8	$F_{(4.000, 8.000)} = 5.05$	-12.65 to -1.347	0.0167

Table 1: Statistically significant data of the experiment of success of hatching of UV-irradiated embryos in comparison to the non-irradiated (control) embryos (ANOVA, followed by Post Hoc Tukey's tests ($p < 0.05$)).

Treatment	DF	F	CI (95%)	P
Control vs. UVB 05 kJ/m^2	8	$F_{(4.000, 8.000)} = 221.7$	-84.08 to -61.10	< 0.0001
Control vs. UVB 1 kJ/m^2	8	$F_{(4.000, 8.000)} = 221.7$	-76.84 to -53.86	< 0.0001
Control vs. UVB 2 kJ/m^2	8	$F_{(4.000, 8.000)} = 221.7$	-87.61 to -64.64	< 0.0001
Control vs. UVB 4 kJ/m^2	8	$F_{(4.000, 8.000)} = 221.7$	-100.8 to -77.79	< 0.0001
Control vs. UVA 25 kJ/m^2	8	$F_{(4.000, 8.000)} = 23.27$	-87.82 to -39.14	0.0001
Control vs. UVA 50 kJ/m^2	8	$F_{(4.000, 8.000)} = 23.27$	-52.99 to -4.312	0.0220

Table 2: Statistically significant data of the experiment of tadpoles' mortality rates induced by both UVB and UVA radiation (ANOVA, followed by Post Hoc Tukey's tests ($p < 0.05$)).

Figure legends

Figure 1: Daily profiles of solar UVB (**A**) and UVA (**B**) radiation incidence upon the breeding site of *H. curupi* (black line) and in a converted area (deforested area; gray line) inside TSP.

Figure 2: Success of hatching after the exposure of embryos to different doses of UVB (**A**) and UVA (**B**) radiation. *Statistically significant difference in comparison to the respective control (bars represent the standard deviation of the average, $p < 0.05$).

Figure 3: Embryonic mortality rates induced by UVB (**A**) and UVA (**B**) radiation. *Statistically significant difference from the respective control (bars represent the standard deviation of the average, $p < 0.05$).

Figure 4: Impact of UVB (**A**) and UVA (**B**) radiation on mass (g) of hatched *H. curupi* tadpoles. *Statistically significant difference from the respective control (bars represent the standard deviation of the average, $p < 0.05$).

Figure 5: UVB-induced malformations on hatched *H. curupi* tadpoles. (**A**) Normal tadpole (non-irradiated control group), (**B** and **C**) tegument hyperplasia in UVB-hatched tadpoles, and (**D**) kinky tail (sipinal curvature) in UVB-hatched tadpole.

Figure 6: Mortality rates induced by UVB (**A**) and UVA (**B**) radiation on hatched tadpoles after 240 h. *Statistically significant difference from the respective control (bars represent the standard deviation of the average, $p < 0.05$).

Figure 7: Evaluation of forest canopy throughout a period of 20 years at the TSP’s area. Integrated satellite images concerning the periods from 1990-2000 (**A**), and 2000-2010 (**B**); percentage of preserved area in each analyzed decade (**C**). Red spots indicate open areas inside the forest due to timber activity and the green area represents the preserved forest area.

Figure 1

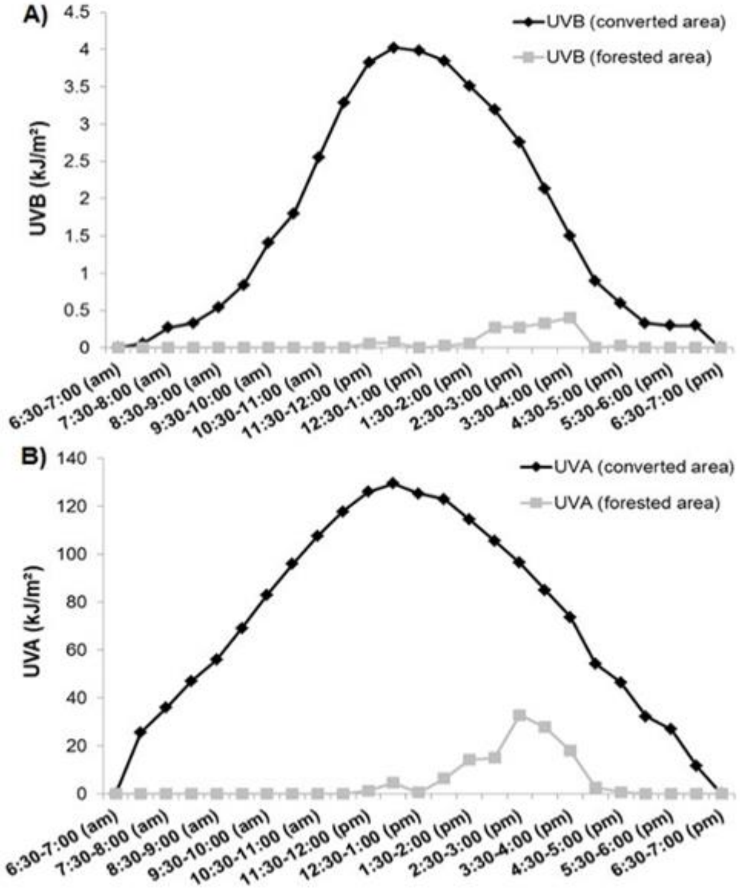

A)
UVB (kJ/m²)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
UVB (converted area)
UVB (forested area)
6:30-7:00 (am)
7:30-8:00 (am)
8:30-9:00 (am)
9:30-10:00 (am)
10:30-11:00 (am)
11:30-12:00 (pm)
12:30-1:00 (pm)
1:30-2:00 (pm)
2:30-3:00 (pm)
3:30-4:00 (pm)
4:30-5:00 (pm)
5:30-6:00 (pm)
6:30-7:00 (pm)
B)
UVA (kJ/m²)
140
120
100
80
60
40
20
0
UVA (converted area)
UVA (forested area)
6:30-7:00 (am)
7:30-8:00 (am)
8:30-9:00 (am)
9:30-10:00 (am)
10:30-11:00 (am)
11:30-12:00 (pm)
12:30-1:00 (pm)
1:30-2:00 (pm)
2:30-3:00 (pm)
3:30-4:00 (pm)
4:30-5:00 (pm)
5:30-6:00 (pm)
6:30-7:00 (pm)

Figure 2

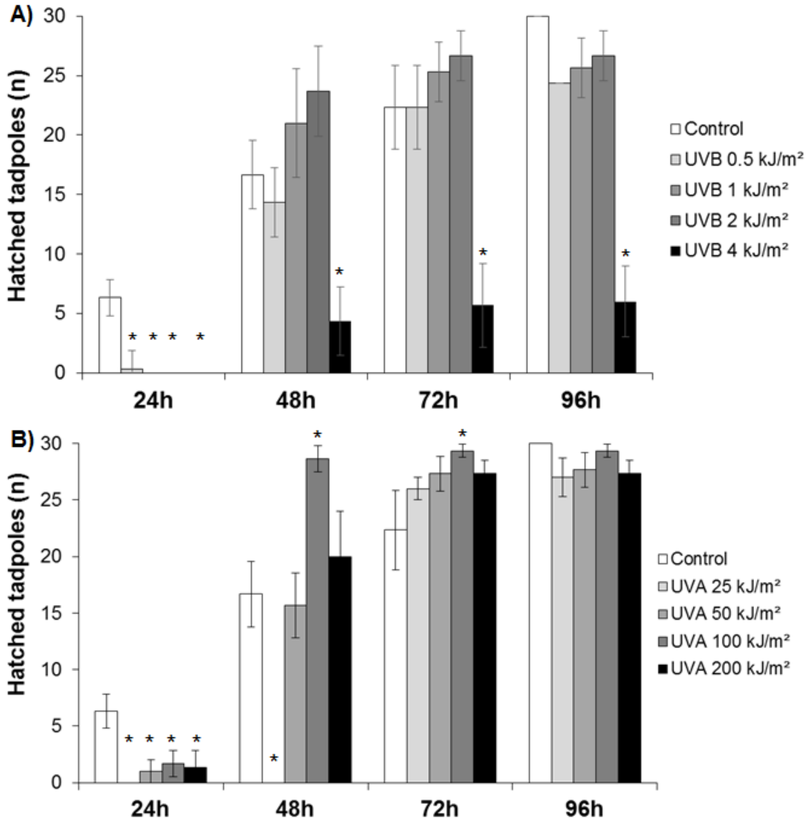

A)
Hatched tadpoles (n)
0
5
10
15
20
25
30
24h
48h
72h
96h
*
□ Control
□ UVB 0.5 kJ/m²
■ UVB 1 kJ/m²
■ UVB 2 kJ/m²
■ UVB 4 kJ/m²
B)
Hatched tadpoles (n)
□ Control
□ UVA 25 kJ/m²
■ UVA 50 kJ/m²
■ UVA 100 kJ/m²
■ UVA 200 kJ/m²

Figure 3

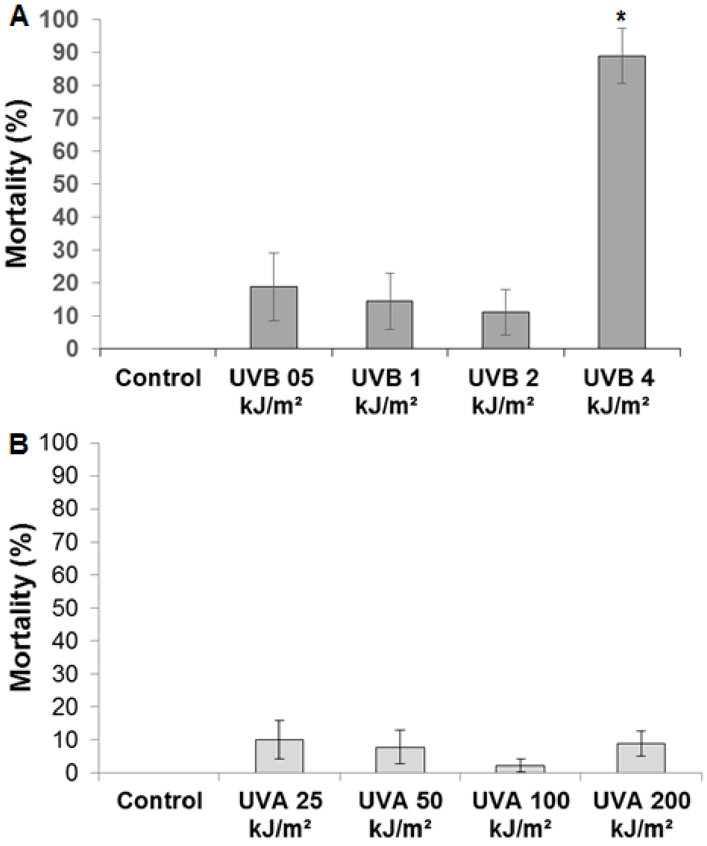

A
Mortality (%)
100
90
80
70
60
50
40
30
20
10
0
*
Control
UVB 05 kJ/m²
UVB 1 kJ/m²
UVB 2 kJ/m²
UVB 4 kJ/m²
B
Mortality (%)
100
90
80
70
60
50
40
30
20
10
0
Control
UVA 25 kJ/m²
UVA 50 kJ/m²
UVA 100 kJ/m²
UVA 200 kJ/m²

Figure 4

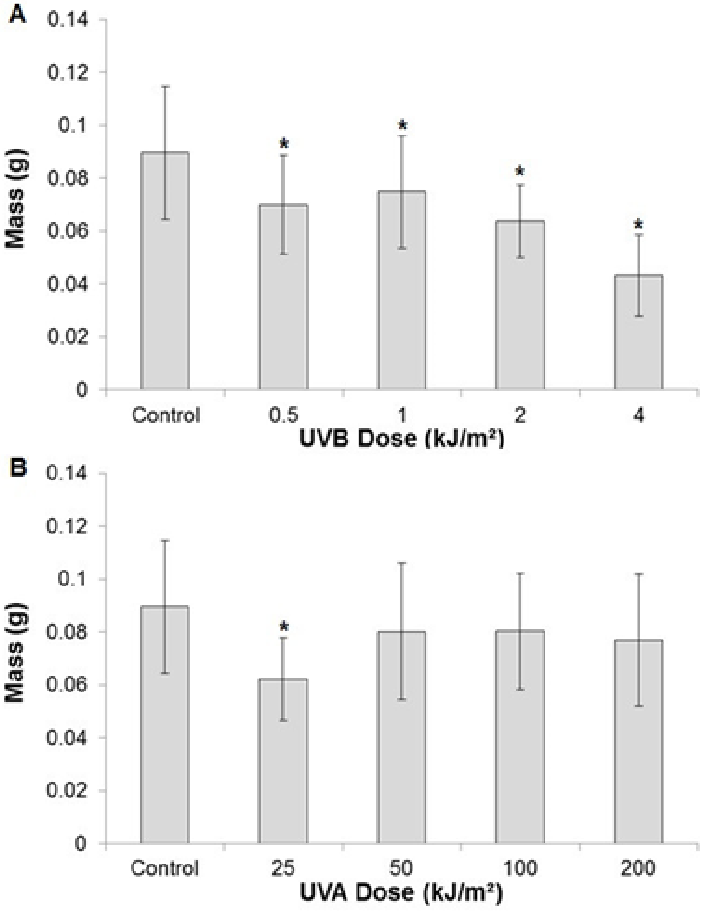

A
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Mass (g)
*
*
*
*
Control
0.5
1
2
4
UVB Dose (kJ/m²)
B
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Mass (g)
*
Control
25
50
100
200
UVA Dose (kJ/m²)

Figure 5

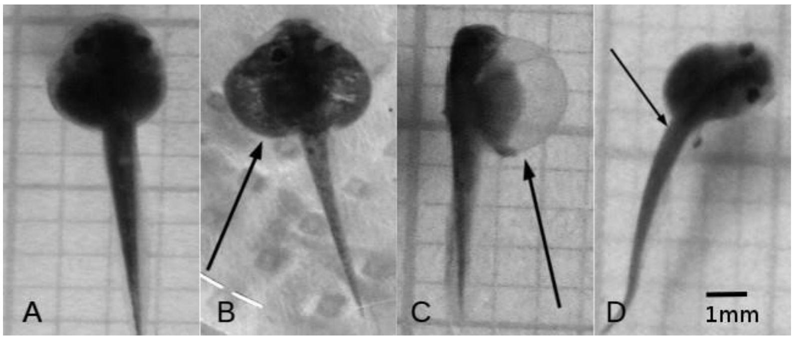


Figure 6

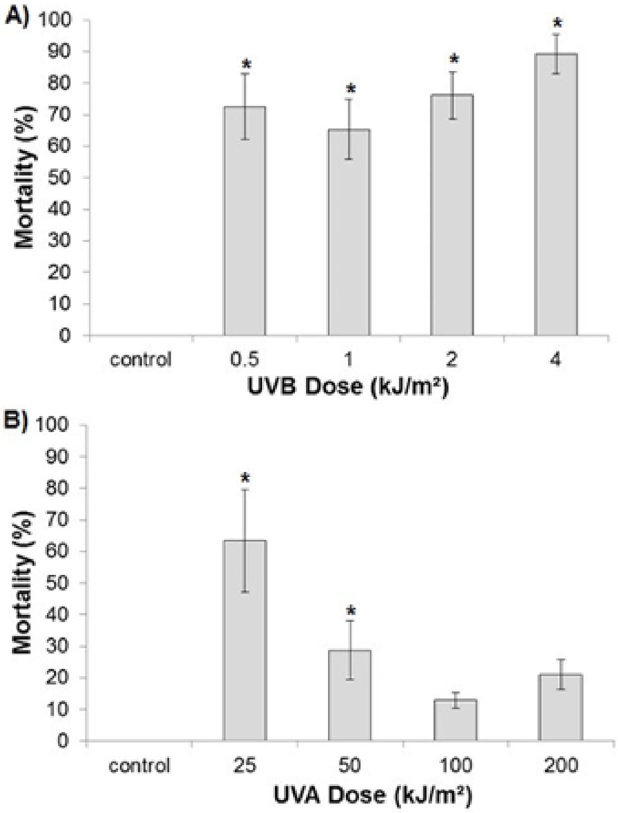
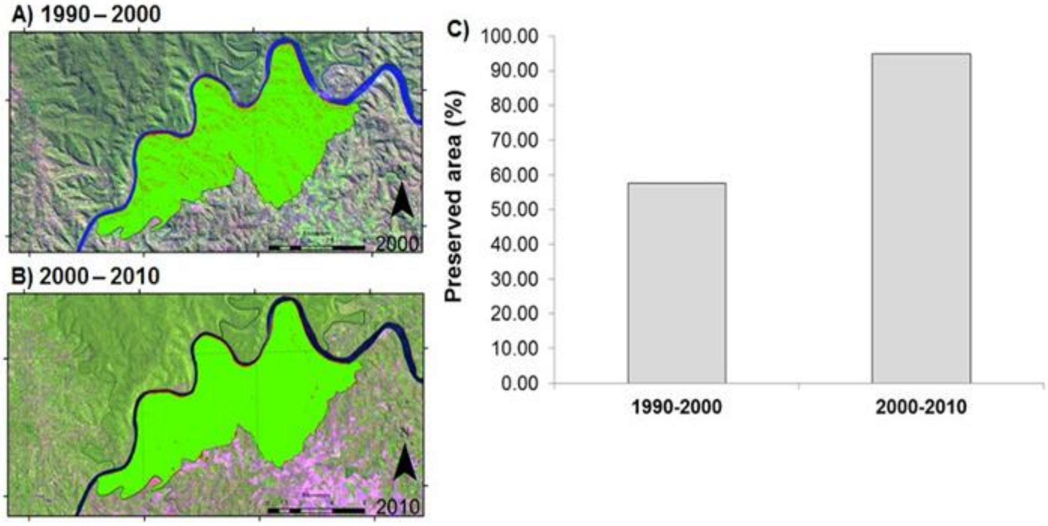

A)
Mortality (%)
100
90
80
70
60
50
40
30
20
10
0
*
*
*
*
control
0.5
1
2
4
UVB Dose (kJ/m²)
B)
Mortality (%)
100
90
80
70
60
50
40
30
20
10
0
*
*
control
25
50
100
200
UVA Dose (kJ/m²)

Figure 7


A) 1990 – 2000
2000
B) 2000 – 2010
2010
N
C)
Preserved area (%)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
1990-2000
2000-2010

Capítulo II

Anuran assemblages along a forest-agricultural gradient: nested patterns or turnover?

Community Ecology

Anuran Assemblages Along a Forest-Agricultural Gradient: Nested Patterns or Turnover?

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Abstract. The conversion of tropical environments into agricultural land has become a global issue since the protected areas are in general restricted to a small portion inside the Conservation Units inserted in pasture or plantation matrix. The fragmentation and the habitat loss is associated to many other cofactors, which is leading the decline of several taxa and to widespread extinction events. The amphibians are the most threatened group among the vertebrates, given their physiological and distributional limitations, they are facing rates of extinction higher than the background. Our main objective is to understand what is pattern of organization of the anuran communities present on an environmental gradient that ranges from an agricultural matrix to a well-preserved forest. We found 17 anuran species belonging to five families, and from these, just one species occurred exclusively in agricultural land. We found that the turnover component of the beta diversity is more important than the nested pattern found. Indicating that some selective process is acting and shaping the communities, given de absence of a set of species at the converted area, probably driven by habitat loss. Our results show that cultivation has disturbed or replaced habitats needed by the anuran, leading to an impoverishment of the communities.

Keywords. Atlantic Rainforest; Conservation unities; Converted area; Forested area; Habitat loss.

INTRODUCTION

The conversion of tropical rainforests into pasture and cropland has become a serious environmental issue, and the challenges on management necessary to defining the conservation units has also become an social problem (Brown et al. 1992, Stagl et al. 2014). The deforestation process is not stopping, instead, it is growing and changing the spatial patterns, accelerating the loss of forested areas (Maes 2004) and impacting severely the local biodiversity. The Brazilian Atlantic Rainforest is a hotspot of biodiversity, which in the three past decades lost 88% of its original area (Myers et al. 2000) due to the suppression/changes of the native forest by local anthropogenic activities (Eterovick et al. 2005, Carnaval et al. 2006). Regarding this growing threat on the biodiversity only ten years ago (after 14 years of evaluation at the congress), the Atlantic Rainforest law project (law 11.428, December, 22 of 2006) was finally signed by the former president creating a new approach for the conservation of the biome, which regulates the use and exploitation of its forest remnants and natural resources.

Actually, still the protected environments like the Brazilian Atlantic Rainforest, restricted to small portions of forest inside Conservation Units (CU's), and as happened in other places thought the world, are administered by severely restricted funding from politically and administratively weak governments (Peres and Terborgh 1995). As stated by Iop et al. (2011), the high fertility of the soil and the extensive plains are conducive to agriculture and cattle breeding, being these the major threats, also associated with the economic interest on the extraction of wood that (used to) occur in many of these places (Murphy and Lugo 1986, Prado and Gibbs 1993).

Understanding how ecological and evolutionary processes affects the structure of the ecological communities brings important implication in conservation politics

(Patterson 1990) since the world is passing through an era where fragmentation and habitat loss are associated with other cofactors leading the decline events and thus, local and widespread extinctions of several taxa (Bergman and Landin 2002, Maes 2004, Strauss et al. 2010, Kurten 2013, Dirzo et al. 2014). After extensive reviews (see Stuart et al. 2004, McCallum 2007, Hayes et al. 2010, Blaustein et al. 2011) the scientific community also showed that these events are far more widespread and severe than would be expected under normal demographic variation (Pounds and Crump 1994, Pounds et al. 1997, 2006, Stuart et al. 2004).

According to the International Union for Conservation of Nature (IUCN), the amphibians group, is the most threatened among all the vertebrates, even more than birds and mammals (Blaustein et al. 2011) and given their physiological and distributional limitations, are facing rates of extinction 211 times higher than the background rates of extinction (Blaustein et al. 2011). The habitat modification is one of the best documented causes of amphibian population declines or extinction. It acts diminishing abundance and diversity of amphibians due processes like removal and/or clearcutting the mature vegetation (Ash 1997, Hecnar and M’Closkey 1996). The deforestation can expose amphibians to altered microclimatic factors like soil compaction, desiccation, and reduction in heterogeneity of the habitat (Welsh 1990, Ash 1997). It may also enhance the exposition of aquatic amphibians at streams to UV radiation by reducing woody debris (Bury and Corn 1988). Although, as stated by Ash (1997) and Alford and Richards (1999), natural populations can recover its earlier stages as forests regain its maturity, but only if these areas of forests are not yet replaced with monocultures.

Regarding to these rising problems, we have evaluated a general presumption that (H1) environmental gradient is an important mechanism influencing the organization and differentiation of the anuran communities. Therefore, we have predicted that (H1a) the anuran richness is higher at more heterogeneous landscape (i.e. in the native forest) due to this presents higher number of reproductive habitats and an environmental quality enhanced (Griffin et al. 2009, Vasconcelos et al. 2009, Iop et al. 2012), contrasting the well accepted presumption that majority of species would prefer breeding habitats in agricultural landscapes given their generalist habits (Duellman 1999, Santos et al. 2007), and also given the importance of the anthropic waterbodies to maintain many anuran species at the pasture/crop matrix (De Marco et al. 2014). We also predict that (H1b) the turnover component of the beta diversity would be the dominant component throughout this environmental gradient, given the lower vagility (Dobrovolski et al. 2012) of the anurans

MATERIALS AND METHODS

Study area description

The study site comprises the two extremes of an environmental gradient characterized by native forested (NF) and agricultural landscape (AL). Both areas are inserted in a region originally characterized by Semideciduous Forest (*sensu* Prado 2000), at the municipality of Derrubadas (Fig. 1; 27°8'44" S, 53°53'10" W; 100-400 m altitude) at the extreme north-western of the state of Rio Grande do Sul, Brazil. The converted portion is an intensively agricultural landscape dominated by soybean, corn and wheat crops covering and cattle pasture with approximately 361.199km^2. The native portion is the largest Atlantic Rainforest conservation unit at the southernmost of Brazil, Turvo’s State Park hereafter PET, which covers 17.491ha and have a perimeter of about 90 km, bordered by the Republic of Argentina and the Brazilian state of Santa Catarina via the Uruguay River (SEMA 2005). The local climate, is characterized as subtropical sub-humid with dry summer (Maluf 2000), have an average temperature of above 22ºC on the warmest month (January) and 13.3ºC at the coldest month (July). The annual rainfall (average) is about 1665 mm, being well distributed throughout the year (SEMA 2005).

Study site selection and pond description

Were selected 38 ponds, distributed along the gradient (19 at the NF portion and 19 at the AL). The ponds selected outside of the park represent anthropic environments characterized by agricultural matrix, whereas the ponds selected inside of the Park are surrounded by forest matrix. In order, to access the information about environment heterogeneity we took measures of three classes of environmental predictors: a) water parameters (pH, temperature, salinity, electric conductivity, and dissolved oxygen), b) pond structure (canopy coverage, percentage of floating and/or emerging vegetation, number of profiles of emergent vegetation, and depth), and c) landscape attributes (distance from CU´s border, presence of agricultural matrix, and number of near buildings).

Anuran community sampling

We conducted two seasons of sampling using the method of "*survey at breeding sites*" (Scott Jr and Woodward 1994). The first sampling effort was conducted at the beginning of the anuran breeding season (September/2013), the middle (December/2013), and the end of the breeding season (March/2014). The second sampling effort was conducted the same way, at September, December and March of 2014/2015, respectively. This sampling effort was chosen to coincide with the breeding season of all anuran species previously recorded at the studied area (see Iop et al. 2011). We visited each pond (in a randomized the sequence to avoid sampling bias) at daytime to collect anurans at larval stage (to increase the probability of species detection), and at nighttime to calling survey (between 20:00 h and 01:00 h). Species recorded just by visual encounters (i.e. no calling individuals) were considered only for richness data. The larvae were identified at species level and all the collected specimens were deposited at the “Coleção Herpetológica da Universidade Federal de Santa Maria” (ZUFSM).

Statistical analysis

To test if ponds in native forest and agricultural landscape differ in structure of anuran communities (i.e. pattern of species abundance) we applied an Analysis of Similarity (one way ANOSIM) (Clarke et al. 2006) on a distance matrix of Bray-Curtis index (Legendre and Legendre 1998) based on the abundance of adult anurans (including only the calling males) previously transformed (by Hellinger transformation), and consider only those with the minimum total of three individuals. In addition, we represented the distance matrix by Non-metric Multidimensional Scaling analysis (NMDS; Legendre and Legendre 2012). After this procedure, we investigated the beta diversity components (Baselga 2010), in order to understand which of these (turnover of nesting pattern) were working on the organization of the anuran community. This approach computes by using pair-wise dissimilarities (distance matrices) and multiple-site dissimilarities, separating the turnover and nestedness-resultant components of diversity.

In order, to test if the anuran community from the AL is a subset of that present in NF we performed a Nesting Test (Baselga 2010, 2012) by constructing a presence-absence matrix where columns and rows were ponds and species (adults and tadpoles), respectively. To quantify the pattern of nestedness, we use the metric based on overlap and decreasing fill (NODF; Almeida-Neto et al. 2008), whose metric assume values from 0 to 100, where higher values indicate more nested assemblages. After this procedure, we performed an analysis of partition of the beta diversity into turnover and nestedness components. This analysis computes three multiple-site dissimilarities accounting for the spatial turnover and the nestedness components of beta diversity, and as well the sum of both values.

We performed an Indicator Species Analysis (ISA) to identify the species or groups of species with highest association/ fidelity to each studied area. This analysis allows us to show if an species is exclusive from one given area (A=1) or/ and if the species is faithful to the place (B=1) (Dufrêne and Legendre 1997, De Cáceres and Legendre 2009, De Cáceres et al. 2010).

We compared total species richness recorded in NF and AL by coverage-based rarefaction and extrapolation (Chao and Jost 2012), available at the iNEXT program (Hsieh et al. 2013). In this method, species richness is estimate by rarefying and extrapolating samples for sample coverage up to a coverage specified by the user, and 95% confidence intervals are calculated by Bootstrap (50 re-samples). This analysis was based on species incidence data (considering records of both, adults and tadpoles). Finally, we undertook a regression analysis of the anuran richness of each community against the environmental variables (see environmental heterogeneity above) transformed by Log x+1, using Generalised Linear Modelling (GLM) with a forward stepwise regression, which provides indication of influences of the environment at multiple scale on anuran community. Additionally, we performed an analysis of partition of variance (Legendre et al. 2005, Jones et al. 2008, Rangel et al. 2010) to provide more information on the importance of each environmental component (water predictor, pond structure, and landscape predictor), and possible interactions.

RESULTS

In this study, we found 17 anuran species occurring at the studied ponds (one of them is an exotic species), belonging to six families: Bufonidae (2), Hylidae (6), Leptodactylidae (6), Microhylidae (1), Phyllomedusidae (1) and Ranidae (1). Six anuran species (*Leptodactylus fuscus*, *L. mystacinus, Ololygon aromothyella, Phyllomedusa tetraploidea, Rhinella ornata, Scinax fuscovarius,* and *S. perereca*) were recorded only in NF, whereas just one species occurred exclusively in AL (*Leptodactylus gracilis*) (Table 1).

The Analysis of Similarity showed that the anuran communities from native forest and agricultural landscape are significantly, but weakly different (R = 0.142 P<0.05) in their species composition, resulting in high overlap of AL and NF ponds at the NMDS ordination (Fig. 2). On the other hand, the nesting analysis showed a nesting pattern, since the anuran community of agricultural landscape (AL) was an impoverished sub-set of the richest community recorded in native forest (NF) (Matrix fill = 0.35, NODF = 62.44 P<0.05). The partition of the beta component of diversity showed that within both communities the component "*turnover*" was higher than the "*nested-resultant*" component (Fig. 3.)

We found that the average of anuran species richness per ponds was 6.68±2.88 (min = 3 and max= 14 species) in native forest community (NF), while the richness average was of 5±2.49 (min = 3 and max= 10 species) in agricultural landscape (AL). Moreover, the total richness differed between the studies areas, since NF presented higher species richness than AL (Fig. 4). Furthermore, the Species Indicator Analysis showed four anuran species highly associated with the ponds from NF, and just one species with AL ponds, respectively (Table 3).

When NF and AL were considered together, anuran species richness increased with water temperature and decreased with the presence of crops (soybeans, corn and wheat). In separated, the anuran species richness in NF increased with water's dissolved oxygen, with the distance from the CU's nearest border, and with the canopy coverage. However, the anuran species richness in the agricultural landscape was influenced (positively) only by the distance from the CU's nearest border. The partition of variance for NF showed that dissolved oxygen and canopy coverage (explanation of 21.4% and

24.9%, respectively) were not significant (P>.05), whereas the distance from the nearest border explained 56.6 % (P<.05) of the variance of species richness (Table 4).

DISCUSSION

Herein we reported that anuran communities of native forest and anthropic landscape differed widely regarding ecological metrics such species richness, species contribution, and the major environmental descriptors regulating pattern of habitat usage (H1). We found both, some anuran species with restrict distribution (i.e *Ololygon aromothyella* and *Phyllomedusa tetraploidea*) associated to the Atlantic Rainforest, as well as some species widely distributed throughout entire South America (Iop et al. 2012). The agricultural landscape presented lower anuran species richness (H1a) and was characterized as a poorly subset of the anuran community from the NF (H1b). This poor subset community is composed by generalist species, with higher dispersal capacity (e.g. *Rhinella* group), resistant to dissecation, less susceptible to the environmental modifications (Leandro and D'Almeida 2005, da Cunha Bitar et al. 2015), better adapted to hydrological stress, as well as high environmental enhances of temperature (Moraes et al. 2007) and other disturbances occasioned by the human activities. These effects were linked to some know regulators of richness composition and diversity (H1c) of vertebrate communities, local predictors and landscape predictors associated to water conditions and to landscape configuration (Fischer and Lindenmayer 2005, Vasconcelos et al. 2009, Iop et al. 2012).

About the beta diversity components, there is not a single mechanism influencing the loss of species and the changes on species composition. There are many mechanisms that can explain the nested patterns of anuran communities (Almeida-Gomes et al. 2016) and also of other animal and plant *taxa* (Wright et al. 1998, Wang et al. 2010). Here we found that there are local and landscape predictors leading the impoverishment of AL communities making them a poor subset of NF, where few species tended to be present along the entire studied gradient, and other species, tended to be exclusive from native sites (Hecnar and M'Closkey 1997). Despite the existence of a nested organization on the gradient, we saw that the main generator of beta diversity in both anuran communities is the turnover component, which implies, in general, in high regional diversity (Machado et al. 2012) and define the changes on species composition as the main structural component, but the ability of colonizing or not a given place is linked to the environmental function of the species (de Souza Queiroz et al. 2015).

This kind of pattern suggests the existence of ecological processes (i.e selective extinction) responsible for regulating the presence or absence of anuran species at these different communities along the gradient (Bittencourt-Silva and Silva 2014, Seshadri 2014). Events like habitat-split and habitat loss can act negatively at population size, structure, and distribution of species (Becker et al. 2007, 2010) leading to isolation (and inbreeding), genetic erosion, and generating local extinctions (Patterson 1984, Wright et al. 1998, Dixo et al. 2009, Bittencourt-Silva and Silva 2014). The same pattern can be seen at many other vertebrate communities, where similar events leaded the disappearance of some species in sequence in a given area (Fischer and Lindenmayer 2005, Rocha et al. 2014).

Dissecting the environmental factors that influenced on the enhance or the lowering of richness in both sampled communities (AL and NF), we highlight that the presence of an agricultural matrix near the pond exerted a negative influence on it. The presence of monocultures is known by disturb and replace habitats (Anderson and Arruda 2006, Pillsbury and Miller 2008, Russell and Downs 2012). This conditions may explain, in the case of the present study, the absence or the lower number of anuran species that vocalize perched on tree branches or have specialized reproductive modes (Newbold et al. 2015). The deforestation process needed to establish a pasture or a cultivation field results in loss of habitat quality and decrease the number of microhabitats for reproduction, foraging and/or oviposition (Urbina-Cardona et al. 2006). It also changes the availability of the vertical strata (provided by forest environments) as well the thermal control, and the incidence of UV radiation which can be determinant to diversity loss (Hof et al. 2011, Isaacs Cubides and Urbina Cardona 2011, Schuch et al. 2013, 2015).

Regardless the effects on the physic-chemical variables of the environment, the forest logging or the conversion of a preserved area into an agricultural matrix may impose other limiting factors to the establishment of anurans. Historical data already suggested that the amphibians are susceptible to dispersal barriers (Vos and Chardon 1998, Trenham and Shaffer 2005) and, usually, respond negatively to it. These non-suitable conditions are usually found at the converted areas (Mader 1984, Babbitt et al. 2006) and act limiting the distribution of species less plastic turning these kind of matrix practically impermeable, and, as showed by other studies, they act as substantial barriers for small juveniles due the harsh environmental conditions (Rothermel and Selmitsch 2002, Rothermel 2004). Furthermore, the long-term survival of populations or communities in a given place is hardly influenced by the ability of rapid dispersal of juveniles along the environment and the breeding sites (Rothermel and Selmitsch 2002, Rothermel 2004), explaining the enhance of anuran richness at the ponds located in a higher distance from the border to the interior of the conservation unit.

Since habitat preferences vary by species, the agriculturally-driven habitat loss is a detrimental factor to anurans (Cushman 2006, Fahrig 2010), this habitat change could exclude individuals that benefits on some specific portion of the habitat. The prevalence of open areas at a previously forest ecosystem could restring the occupancy of it, sometimes by segregating the use of microhabitat (i.e. the reduction of occurrence of treefrogs), making harder the process of colonization and dispersion. On the other hand, these changes may also benefit species that evolved adapted to occur or at least survive in open areas being by their physiological adaptations or being by their specialized reproductive modes (i.e. leptodactylids and with underground oviposition sites). Anyway, the suppression of native forest can change the communities' composition, creating a desbalance on the occurrence of some species (see *Physalaemus* aff. *gracilis*, *Scinax perereca* and *Rhinella ornata*) that have some degree of sensitivity to environmental changes (Dixo et al. 2009, Moreira et al. 2015).

As happened in other studies, the landscape characteristics have explained the pond occupancy more than the local characteristics (Mazerolle et al. 2005, Smallbone et al. 2011), showing that the surroundings of the waterbodies may play an important role in the distribution of the adult anurans and also the larval stages (Rothermel and Selmitsch 2002, Rothermel 2004, Provete et al. 2014). Although these kind of environmental descriptors are of large-scale, they can alter even the primary productivity and the water chemistry (e.g. temperature, conductivity, and dissolved oxygen) being of paramount importance on anuran development since the deforestation processes, while modifies environmental factors also acts at the interspecific interactions like, predation, parasitism and competition (Harper et al. 2005).

To understand the processes like turnover and nestedness patterns is crucial to determine how the communities are organized (Baselga 2010). In addition to this information, understand which of these components is dominant in the community also helps to answer important questions about the conservation status both of the species and of the habitat. Here we saw that the turnover component is the main generator of beta diversity of our tested communities, being the substitution of species more important than the loss of species at the gradient, a pattern related to the nestedness component that have a low importance, but it is still an existent pattern. In this way, it is also important to know which of the environmental variables is responsible by the generation of these kind of pattern. In general, we saw that the changes at the composition of species is mainly related with the amount of foraging and shelter places an as well, the availability of breeding sites, remarking the importance of the maintenance of the protected areas and the connectivity between them, a role played by the ponds located at the agricultural landscape.

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TABLES

Table 1. Anuran species (based on adults and larvae) recorded during two consecutive breeding seasons (2013/2014 and 2015/2016) in ponds of the Parque Estadual do Turvo and adjacencies (native forest and agricultural landscape), southernmost Brazilian Atlantic rainforest, north-western of Rio Grande do Sul state, Brazil

Family /Species	**Native Forest**	**Agricultural landscape**
Bufonidae		
Rhinella icterica (Spix, 1824)	+	+
Rhinella ornata (Spix, 1824)*	+	
Hylidae		
Dendropsophus minutus (Peters, 1872)	+	+
Hypsiboas faber (Wied-Newied, 1821)	+	+
Ololygon aromothyella (Faivovich, 2005)	+	
Scinax fuscovarius (Lutz, 1925)	+	
Scinax granulatus (Peters, 1871)	+	+
Scinax perereca Pombal, Haddad and Kasahara, 1995	+	
Phyllomedusidae		
Phyllomedusa tetraploidea Pombal and Haddad, 1992	+	
Leptodactylidae		
Leptodactylus fuscus (Schneider, 1799)*	+	
Leptodactylus gracilis (Duméril and Bibron, 1840)		+
Leptodactylus latrans (Steffen, 1815)	+	+
Leptodactylus mystacinus (Brumeister, 1861)	+	+
Physalaemus aff. *gracilis* (Boulenger, 1883)	+	+
Physalaemus cuvieri Fitzinger, 1826	+	+
Microhylidae		
Elachistocleis bicolor (Guérin- Méneville, 1838)	+	+

Ranidae		
Lithobates catesbeianus (Shaw, 1802) ***	+	+
Species richness by area	**16**	**11**
Total species richness	**17**	

* Less than three individuals (not included at the analysis), ***Invasive exotic species

Table 2. Beta diversity components from anuran communities of native forest and agricultural landscape sampled in ponds during two consecutive breeding seasons (2013/2014 and 2015/2016) at Parque Estadual do Turvo and adjacencies, southernmost Brazilian Atlantic rainforest, north-western of Rio Grande do Sul state, Brazil. β_{sim} is the turnover component (Simpson dissimilarity), β_{sne} is the nestedness component (Sorensen dissimilarity), and β_{sor} is the overall value of beta diversity (Sorensen dissimilarity).

β diversity component	**Native Forest**	**Agricultural landscape**	**Both communities**
Turnover (β_{Sim})	**0.71**	**0.69**	**0.83**
Nestedness-resultant (β_{Sne})	**0.12**	**0.15**	**0.07**
Total dissimilarity (β_{Sor})	**0.83**	**0.84**	**0.91**

*Table 3. **Species indicator analysis (ISA) based anurans sampled in ponds during two consecutive breeding seasons (2013/2014 and 2015/2016) of native forest (NF) and adjacent agricultural landscape (AL) at the Parque Estadual do Turvo and adjacencies, respectively, southernmost Brazilian Atlantic rainforest, north-western of Rio Grande do Sul state, Brazil.***

Species	**Indicative Value**	**Group**	***P* Value**
Leptodactylus gracilis	0.60	AL	0.00
Physalaemus aff. *gracilis*	0.75	NF	0.00
Rhinella ornata	0.60	NF	0.00
Scinax perereca	0.68	NF	0.00

*Table 4. **Generalized regression models from the environmental and spatial attributes with anuran richness recorded in ponds from native forest and agricultural landscape sampled during two consecutive breeding seasons (2013/2014 and 2015/2016) at the Parque Estadual do Turvo and adjacencies, respectively, southernmost Brazilian Atlantic rainforest, north-western of Rio Grande do Sul state, Brazil.***

	Both tested Communities			
	Sum of Squares	**df**	**F**	***P***
Water temperature (ºC)	0.26	1	5.09	0.03
Presence of agriculture	0.22	1	4.15	0.04
	Native Forest			
Dissolved Oxygen (mg/l)	0.14	1	4.63	0.04
Canopy coverage (%)	0.20	1	10.98	0.00
Distance from border (+m)	0.37	1	22.20	0.00
	Agricultural landscape			
Distance from border (-m)	0.30	1	5.25	0.03

FIGURE CAPTIONS

Figure 1. ***Study site at the southernmost Brazilian Atlantic rainforest, north-western of Rio Grande do Sul state, Brazil. Circles indicate the ponds sampled in the native forest of Parque Estadual do Turvo and agricultural landscape of surrounds.***

Figure 2. ***Ordination (by NDMS) of the ponds sampled regarding anuran communities of native forest and agricultural landscape sampled in ponds during two consecutive breeding seasons (2013/2014 and 2015/2016) at Parque Estadual do Turvo and adjacencies, southernmost Brazilian Atlantic rainforest, north-western of Rio Grande do Sul state, Brazil. ● = native forest; ○ = agricultural landscape.***

Figure 3. ***Beta diversity components from anuran communities of native forest and agricultural landscape sampled in ponds during two consecutive breeding seasons (2013/2014 and 2015/2016) at Parque Estadual do Turvo and adjacencies, southernmost Brazilian Atlantic rainforest, north-western of Rio Grande do Sul state, Brazil.*** β_{sim} ***is the turnover component (Simpson dissimilarity),*** β_{sne} ***is the nestedness component (Sorensen dissimilarity), and*** β_{sor} ***is the overall value of beta diversity (Sorensen dissimilarity).***

Figure 4. ***Compared total species richness recorded in NF and AL by coverage-based rarefaction and extrapolation (50 re-samples).***

Figure 1

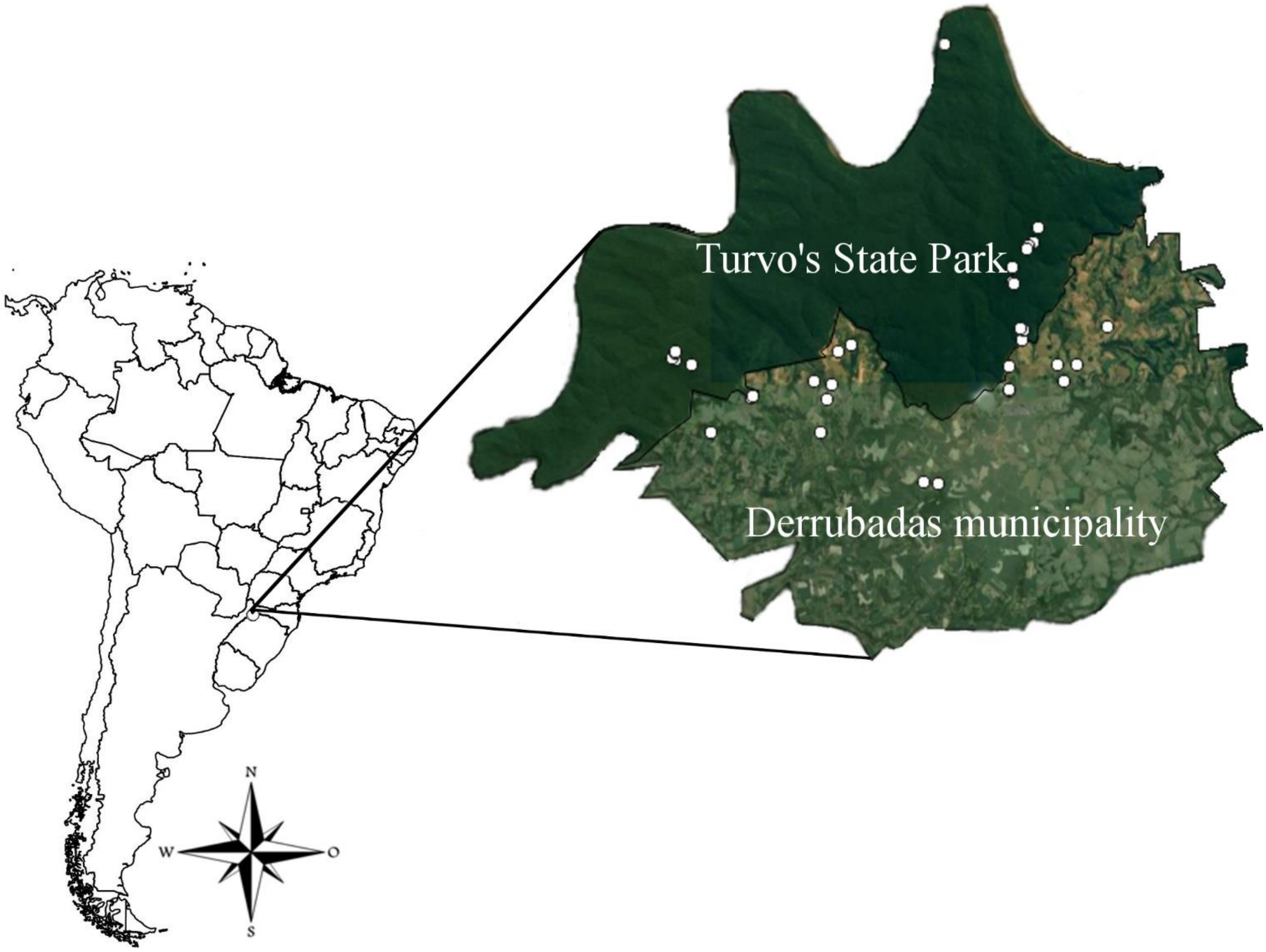

Figure 2

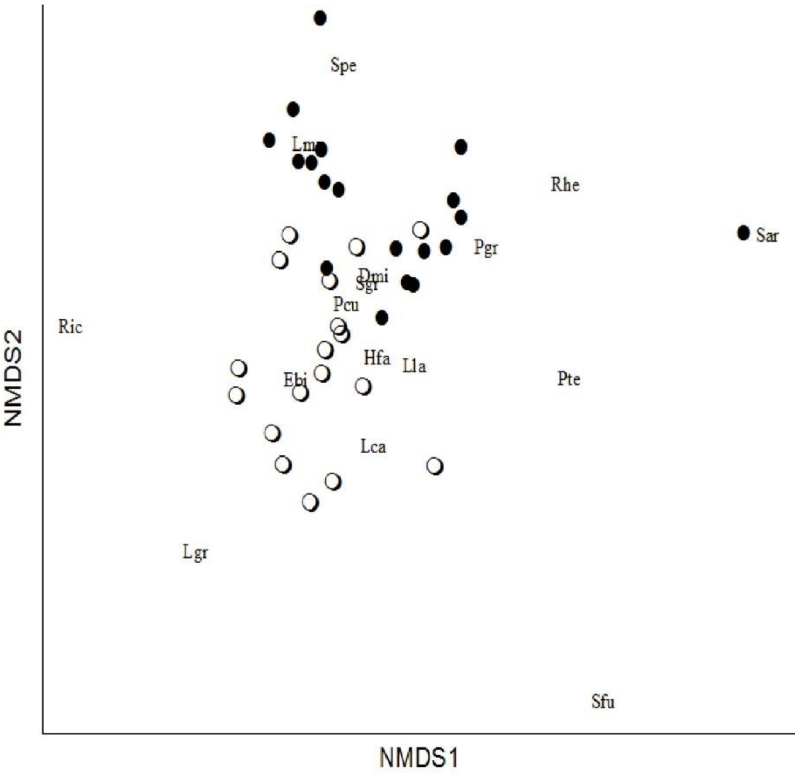
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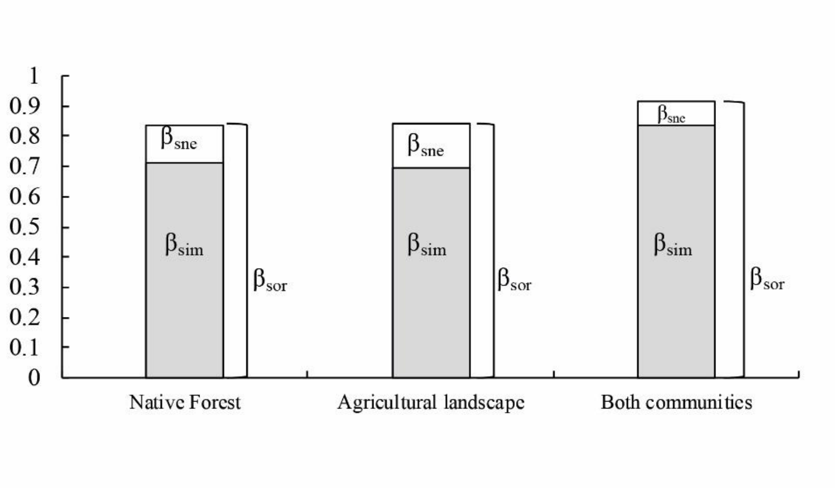
Figure 3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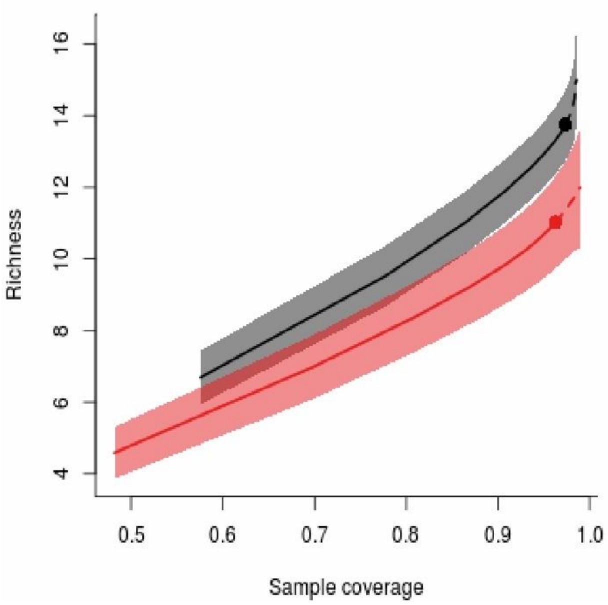
Native Forest
Agricultural landscape
Both communities

Figure 4



Capítulo III

Changes on anuran tadpole functional diversity along an environmental gradient at the southernmost Atlantic Rainforest remnant

Changes on anuran tadpole functional diversity along an environmental gradient at the southernmost Atlantic Rainforest remnant

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Keywords: Anuran; Ecosystem function; Conservation Unities; Tadpoles

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Abstract

For decades the ecologists have been used the same methods in order to determine the environmental health. However, the emergence of the functional ecology as a discipline itself have made necessary the coinage of an expression that could classify the organisms, then defining how the functional diversity of a community can shape the entire organization of it. Here we used the anuran larvae to understand the effects of an environmental gradient on the structure and organization of the anuran communities along it, and to access in a realistic form, the actual state of preservation of the largest remnant of Atlantic Rainforest on the Southernmost portion of Brazil. We found that despite the higher diversity of species and the conservation status of the unit, the ecosystem itself is still in danger, given the low number of functional groups and thus a higher number of ecosystemic services that can be lost.

Keywords: Anuran larvae; Ecosystem function; Conservation Unities; Atlantic Forest.

INTRODUCTION

The emergence of the functional ecology as a discipline itself have made necessary the coinage of an expression that could classify the organisms, in order to define the functional diversity of a community and access the species effects on the ecosystems (Woodward and Cramer 1996, Violle et al. 2007). Thus, the functional traits of a given species was usually defined as any characteristic that exert effects on fitness of an organism, being it physiological, morphological behavioural or developmental (Geber and Griffen 2003, Violle et al. 2007). In this way, the very establishment and/or persistence of a given species on an environment, is somehow a response from the biological characteristics under a set of environmental variables (Southwood 1977, Schmid-Hempel 1991, McGill et al. 2006a, Tellería 2016).

A common approach in community ecology is to investigate the usual associations of environmental characteristics and species richness and composition (Diamond 1975, Rojas-Ahumada et al. 2012, Prado and Rossa-Feres 2014), but let aside the contributions of individual traits and its interactions as a response to the environmental characteristics (Elton 1958, Southwood 1977). In fact, even the species (species level) itself can be considered an assembly of individuals with different arrays of behaviour and phenotypic characteristics, which will determine when and where the species can exist and will also determine how they will interact with individuals from other species (McGill et al. 2006b).

There is an emerging view that uses these eco-morphological measures which make possible understand the trait diversity on an assemblage, and by using these tools we can promote better conservation and/or restoration decisions (Cadotte et al. 2011). In this context, studies aiming to measure the species traits variation as a variable to be modified by the shifts on the environmental conditions (and thus, inducing the species composition in a community), have raised a new tool to evaluate the mechanisms that determine the communities' assembly either at modified landscapes and preserved ones (Petchey and Gaston 2002a, Petchey 2003, de Souza Queiroz et al. 2015).

In our study, we worked with three main objectives: (1) to access the functional diversity of the anuran larvae along an environmental gradient, testing the hypothesis that (H1) the functional diversity of the anuran tadpoles would be higher at the borders of the environmental gradient following the premise that the ponds located close to the agricultural areas would provide both specialized traits and generalist traits, enhancing the values of functional diversity. (2) to test if the values of functional diversity obtained were related with the usual environmental descriptors sampled along the gradient, testing the hypothesis (H2) that the functional diversity would be enhanced following an gradient of complexity of the habitats, following the premise that the dynamism of the landscape provided by more complex environments (e.g. higher number of microhabitat, differences on hydroperiod) could trigger differences on individual responses from the species, which generates higher number of functional traits (see Cadotte et al. 2011). (3) to test if the richness values varies (significantly) along the gradient, and if so, would the functional diversity values be related with the richness values?; by testing the hypothesis (H3) that richness would present minimal changes along this gradient, and the functional diversity would not be related to these values

METHODS

Data acquisition and study site

The field surveys were carried out in two years, during the anuran breeding season (two springs and two summers) at the Turvo's State Park (TSP, Figure 1), a Conservation Unit inserted on a Mesophytic Semidecidual Forest (Prado 2000). TSP comprises an area of 17,491 ha, with about 90 km of perimeter, without a buffer zone to diminishes the effects of the agropastoril activity. We gathered information on the species of anuran tadpoles in 19 ponds, selected according to their availability and their distance from the conservation unit border. Given the undulated nature of the landscape, we had to use all the available ponds at the place, some ponds were close to each other and some were distant. These ponds, in general, have different physiognomic characteristics, so we recorded data on environmental descriptors such as: (i) water depth; (ii) distance from TSP's nearest border; (iii) the number of vegetation types in the interior of ponds (floating plants, emergent and underwater plants, trees and bushes); (iv) water temperature, and (v) canopy coverage (%).

Anuran tadpoles were collected with a dip net (mash of 2mm^2) by exploring the available microhabitats at each pond (e.g. among floating vegetation, different profiles of emergent vegetation, clear water), following McDiarmid and Altig (1999). Larvae were immediately euthanatized and fixed in 10% formalin to posterior identification at laboratory. For each of the anuran species recorded, we compiled 13 functional traits (Appendix table 2), chosen to represent relationships with feeding, swimming, habitat use and/or life-history strategies of the anuran tadpoles ecology, as well as we measure 10 morphometric attributes (Altig and McDiarmid 1999, Strauss et al. 2010) from five tadpoles with stages ranging from 33 to 39 (*sensu* Gosner 1960) (see de Souza Queiroz et al. 2015). The collected tadpoles were identified, measured and deposited at the "Coleção Herpetológia da Universidade Federal de Santa Maria" (ZUFSM), Santa Maria, Rio Grande do Sul state, Brazil.

Data analysis

To access the Functional Diversity (*FD*), the Functional Divergence (*Fdiv*) and the Functional Dispersion (*Fdis*) of the anuran community we obtained three data matrix: 1) a trait matrix of the species with the functional traits of the tadpoles; 2) a community composition matrix with presences and absences of the species at larval stages; 3) a matrix with environmental descriptors of the ponds, to test the association of it with the functional measures (see Supplementary Material S1 for tadpole traits and Supplementary Material S2 for environmental descriptors).

To perform the functional analysis, we first reduced the dimensionality and correlations between our continuous data from the morphometric traits (tadpole body forms) in two axes explaining ~80% of the variation by using a Principal Component Analysis (PCA). Then with the reduced matrix of morphometric traits (1) and the community composition matrix (2), we used a flexible distance-based framework to compute a multidimensional functional diversity (*FD*) indices for each sampled pond. We used the function, *dbFD* (Laliberté and Legendre 2010), this method can handle with any distance or dissimilarity measure, any number of traits (binary, continuous, nominal or ordinal) and have no strong influences of outliers. By using this function, we obtained the values of Functional Divergence (*Fdiv*), that varies from -1 to 1, and Functional Dispersion (*Fdis*) that have no upper limit and then, constructed a dendrogram, based on the functional groups (*FGR*) that represents the similarity/dissimilarity among species according to their ecological traits (Petchey and Gaston 2002a; Petchey et al. 2009).

We also tested, by using a Moran's I index (R's *Moran's I function*) the presence or absence of spatial influence on our data and used a GLM (R's *glm function)* with the environmental matrix (3) to test the existence of any relation of the obtained FD measures with the environmental variables. We also used a *t-test* to see if the values of FD are significantly different from each other. All analysis were performed using the software R (R Development Core Team 2011)

RESULTS

Our sampling effort resulted in the record of larvae from 10 anuran species. The species recorded at the 19 sampled ponds throughout the environmental gradient corresponds to approximately 35% of the total number of species registered to Turvo's State Park, and belongs to five anuran families: Bufonidae, Hylidae, Leptodactylidae, Microhylidae, and Phyllomedusidae (Table 1).

Our first analysis showed that, as expected, there is a variation on *Functional Diversity (FD)* measures among each sampled pond at the environmental gradient. The values of *FD* ranged from 0.19 to 0.31, the values of *Functional divergence* ranged from 0.67 to 0.89, the *Functional dispersion* values ranged from 2.0 to 4.6 and the *FGR* showed the number of functional groups per pond, which resulted in the clustering of seven functional groups based on the species traits (Figure 2). Furthermore, the Moran's I index showed the absence of spatial correlation among the ponds ($p > 0.05$), and the GLM showed a positive relation of the enhance of distance from TSP's border towards the interior on values of *FD* (Figure 3).

DISCUSSION

Our main findings here are to show that the anuran larvae sampled in this environmental gradient belongs to communities regulated by low competition on resource use and a high level of niche differentiation, given the high values of Functional Divergence. These findings are supported when we saw that the high values of Functional Diversity are also a signal or a characteristic of assemblages that act more efficiently on resource exploitation, thus competition is not an important force acting at these places. Furthermore, the seven functional groups clustered by the analysis have both biological and ecological sense when thinking on the morphological and behavioural characteristics of the species presented by each group.

The usual changes observed on the landscape structure along environmental gradients can alter many ecosystem functions, by enhancing or diminishing pressures like the biological filters and abiotic conditions, generating different responses by the community. In this way, these environmental modifications can alter, not only the species richness (a measure largely used to infer the environmental health), as found in many other studies with several other groups (Connell 1978, Kirkman et al. 2001, Werner et al. 2007, Nord and Forslund 2015, Barrett et al. 2016), but can also exert important influence on the

Functional Diversity and other features that, in general, maintain the functionality of the communities.

It is known that the stress caused by these environmental changes, natural or not, do not affect all species equally (Tscharntke et al. 2012). As predicted, in natural ecosystems, all species may have the same probability of extinction, however, the ecological interactions may act in favour or prejudice the capacity of some species (Fonseca and Ganade 2001). These ecological interactions, can be seen at species level as an interaction between evolutionary and ecological dynamics, a relation of trait-environment that shape the capacity of a given species in transpose a barrier and establish itself in a given place (Hutchinson 1957). These kind of information is crucial to understand how communities assemblies and the tolerances of each individual or species to the growing modifications of habitat (Urban et al. 2008).

In this way, our results showed crescent values of Functional Diversity towards the inner places of the TSP, and thus showed that the larval community organizes itself given the functional space occupied by those species, which leads to the formation of the functional groups (Tilman 2001), supporting the theory of ecological guilds (Root 1967). In our case of study, the seven functional groups may point to a factor of importance to environmental conservation, the functional redundancy of species. The low number of species present in each functional group with similar ecological functions is preoccupant, since the higher is the number of species present on each group (with similar functions) would ensure the ecosystem’s higher power of resilience to perturbation (Walker 1995, Rosenfeld 2002).

Contrasting the assumptions of Charles S. Elton (1958) on the book *“The ecology of invasions by animals and plants”*, the complexity (higher or lower) of habitat is not leading higher or lower diversity in our environmental gradient. Since we sampled ponds with different hydrological periods, sizes and presence of predators along the gradient, so different levels of complexity. Although, the values found (FD) at the ponds closer to the borders were smaller than the values found at higher distances. In relation to the environment, the ponds closer to the disturbances (human activity, agriculture/ pasture) showed themselves more stable places and this absence of drastic processes of modification may enhanced predation, intra and interspecific, an ecological factor that could limit the diversification of traits (Pearman 1995, Wellborn et al. 1996).

The constant changes, or "instability" of the ponds located at more distant places may act as an environmental pressure, triggering diversification morphological and ecological attributes enhancing FD on these places. This means that these communities are more efficient on resource exploitation which may be the reason of the high values found to Functional Divergence. In this way, the presence of similar species would not be limited by competition given a higher differentiation on niche usage (Morin 2011). For example, differences on time of spawning would produce tadpoles with different sizes, or different swimming behaviours, fins and tails could enhance the performance and consequently, the success in locomotion and avoiding predation at these places (Cronin and Travis 1986, Nomura et al. 2011, Johnson et al. 2015, de Souza Queiroz et al. 2015).

Since our sampled ponds do not respond to competitive pressures, we can expect that the environment also plays a restrictive role, following the Hutchingsonian Niche Theory (Hutchinson 1957) where some species do not showed the necessary morphological characteristics to overcome the restrictions imposed by the habitat and could not stablish or have difficulty to do it in these places (Cornwell et al. 2006). This is the case of species like *Phyllomedusa tetraploidea* which reproductive mode needs a waterbody below the leaves when the tadpoles hatch, or the larvae from the *Leptodactylus fuscus* group that needs a flood on their subterranean chambers to reach the waterbody and complete the life cycle (Haddad and Prado 2005).

The constant anthropic pressures suffered by the conservation unities, the climatic changes an even some natural processes are putting in danger the health and the stability of the environments. These constant modifications or impoverishment of the natural areas do not affect only a restricted number of species, but the entire trophic web and so, the ecosystem functionality. As stated by Cianciaruso et al. (2009) emerging tools that can incorporate the phylogenetic and functional information of the species tend to be better than those traditional measures (Díaz and Cabido 2001, Petchey and Gaston 2006). In addition to this, our results showed that the richness of species do not varies significantly along the gradient, furthermore, the majority of species was abundant at the sampled ponds, with exception of species like *Hypsiboas faber* that occurred only at the ponds located closer to the border, probably given the plasticity of the species in habit both anthropized ponds and natural ones; and *Scinax fuscovarius* that occurred only at the ponds located far from the border, probably given the habit of vocalize perched on tree branches, that were more common in the inner portion of the gradient; and *Elachistocleis* *bicolor* that was poorly represented at the ponds located both at intermediary and longer distances.

Our results show that the TSP, despite of being a large conservation unit still have to deal with the possibility of losses on ecosystemic functions and remarks the importance of the correct application of the environmental laws. When analysing only the anuran larvae community we saw that there are a low number of functional groups, a low number of species inserted in these groups, and thus to lose one species of this community means to put in risk a lot functions that could be of paramount importance to the conservation of the biodiversity.

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Table I

Anuran larvae species list, their respective reproductive modes and the water column position.*

Species	Reproductive mode	Ecological Guild
Bufonidae		
Rhinella ornata	1	Benthic-Neustonic
Hylidae		
Dendropsophus minutus	1	Nectonic
Hypsiboas faber	4	Benthic
Ololygon aromothyella	1	Nectonic
Scinax fuscovarius	1	Nectonic
Scinax perereca	1	Nectonic
Leptodactylidae		
Physalaemus cuvieri	11	Benthic
Physalaemus aff. *gracilis*	11	Benthic
Microhylidae		
Elachistocleis bicolor	1	Nectonic
Phyllomedusidae		
Phyllomedusa tetrapoidea	24	Nectonic

*Reproductive modes following Haddad & Prado (2005).

FIGURE CAPTIONS

Figure 1. Map showing the location of TSP at South America. The green dots represent the location of 19 sampled ponds.

Figure 2. Functional Dendrogram (FGR) with the seven groups of species. *Pte=*Phyllomedusa tetraploidea*, Pcu= *Physalaemus cuvieri*, Pgr= *Physalaemus* aff. *gracilis*, Hfa= *Hypsiboas faber*, Oar= *Ololygon aromothyella*, Dmi= *Dendropsophus minutus*, Sfu= *Scinax fuscovarius*, Spe= *Scinax perereca*, Ebi= *Elachistocleis bicolor* and Ror= *Rhinella ornata*

Figure 3. Graphical representation of the relation between the distance from the nearest border of the TSP and the Functional Diversity of anuran larvae.

Figures

Figure 1.

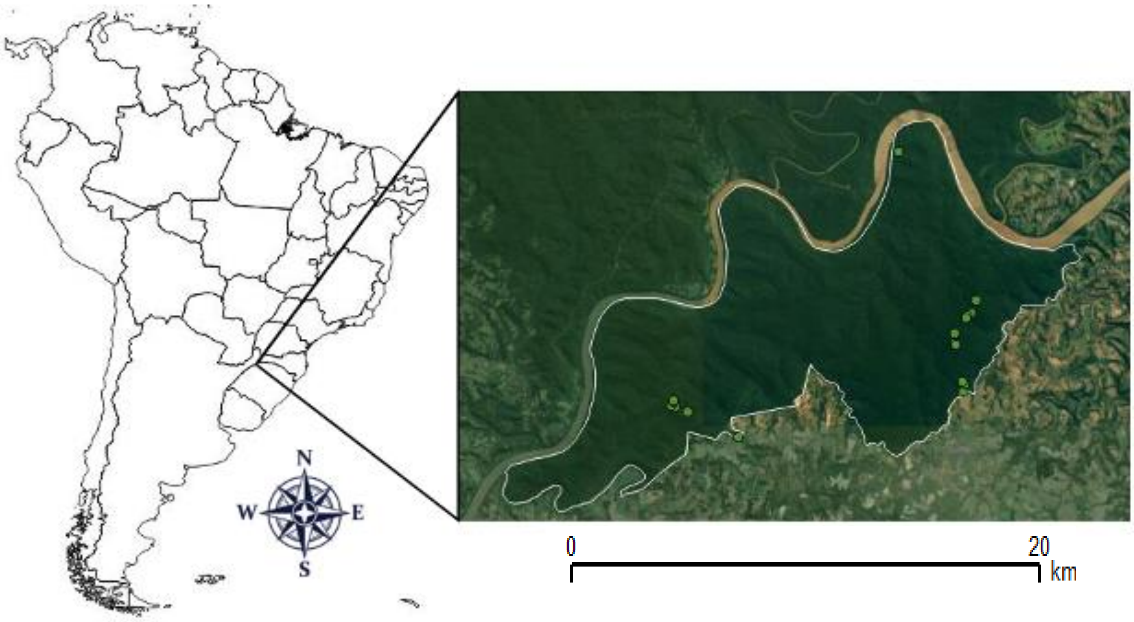


Figure 2.

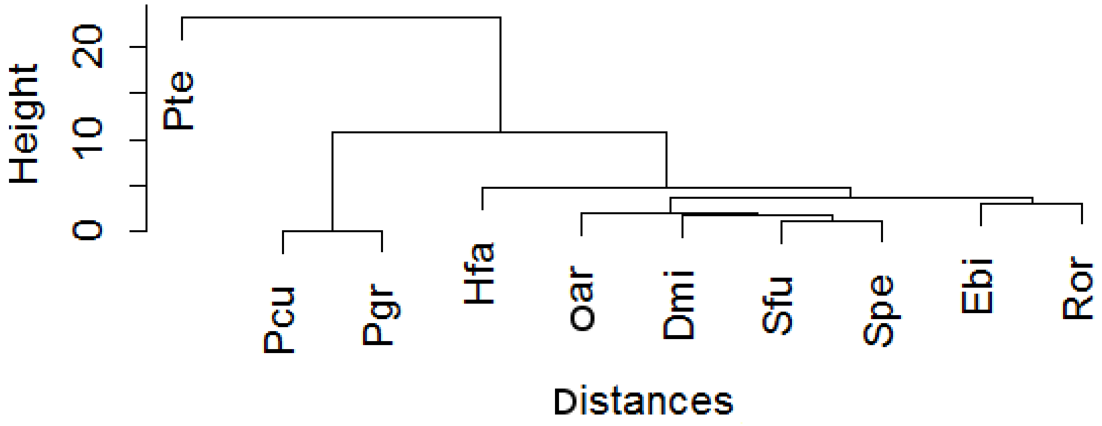
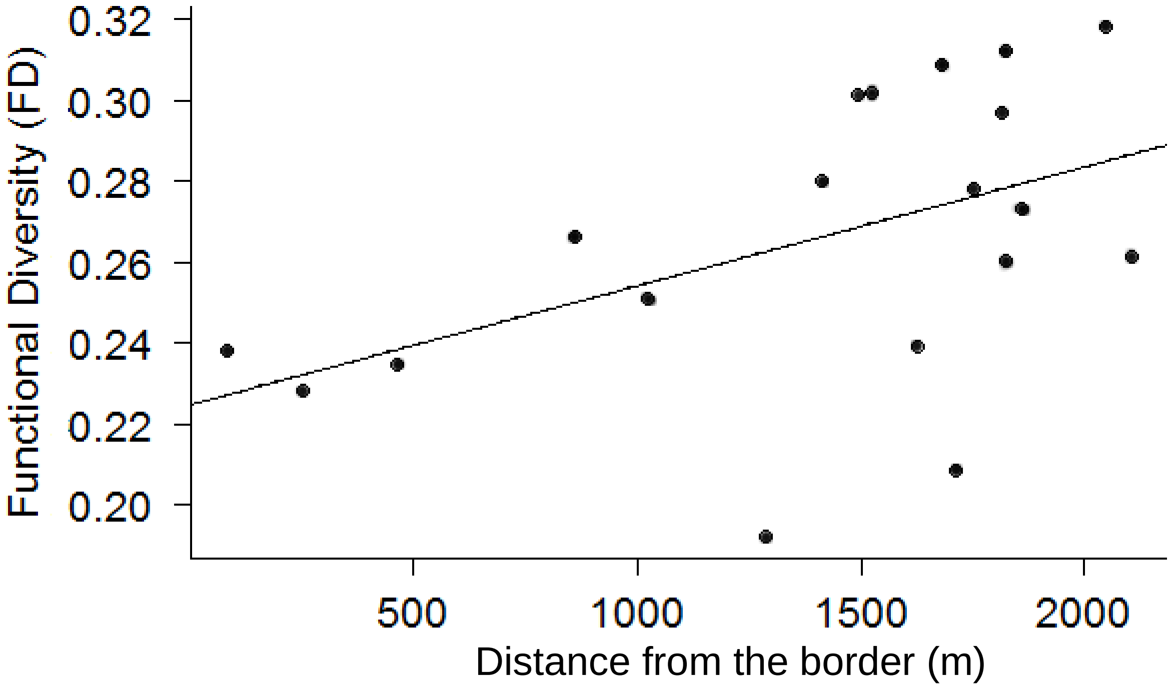

Height
0
10
20
Pte
Pcu
Pgr
Hfa
Oar
Dmi
Sfu
Spe
Ebi
Ror
Distances

Figure 3.


Functional Diversity (FD)
0.32
0.30
0.28
0.26
0.24
0.22
0.20
500
1000
1500
2000
Distance from the border (m)

CAPÍTULO IV

Enhanced phylogenetic diversity: a result of species loss in an agricultural environment

Enhanced phylogenetic diversity: a result of species loss in an agricultural environment

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Abstract

The great environmental changes induced by anthropogenic activities reshaped many ecosystem processes across the globe. The human populations have become persistent in some locations given its activities. Thus, the previously natural landscapes have been turned into mosaics of natural and semi-natural lands embedded into human modified landscape. To understand part of the effects of these landscape modifications on the anuran communities, we aimed to compare pond breeding anuran communities in well-preserved forest with communities in agricultural landscape. We test values of taxonomic, phylogenetic and functional diversity, by analysing four data matrixes containing environmental, phylogenetic, morphologic and abundance information from anuran species along this environmental gradient. Our analysis showed that only the phylogenetic component of the diversity was linked to the changes on the gradient, and that the loss of environmental quality generates an enhance on phylogenetic diversity. We show that the anthropogenic disturbance acts negatively on anuran communities, forcing the species to adapt themselves to act like generalist species in order to survive at the non-healthy places now available, where there were well-preserved forests before the humans change it.

Keywords: Anuran diversity and conservation, landscape conversion, habitat loss, Atlantic Forest.

1. Introduction

The great environmental changes induced by anthropogenic activities are, in general, dated from before the industrial revolution (~1860) [1]. These activities have reshaped many ecosystem processes across the globe since the human populations have become persistent in some places due the enhanced agricultural practices [2]. As a result of this growing phenomena, the previously natural landscapes have been turned into mosaics of natural and semi-natural lands embedded into human modified landscapes [3]. Actually, these mosaic conditions of non-used land represent, in the present, almost 90 percent of the world's tropical forests which are inserted in reserves and parks within agricultural lands [4,5]. Understanding the factors that affect the community assemblies has been the focus of many ecological studies [6–9], since the conversion of the previously undisturbed places are usually allied to unsustainable activities, driving the ecosystem degradation throughout the loss of ecosystem services and the related cascade events [10] causing biodiversity loss across the globe and across spatial, temporal, and organizational scales [11–13].

To understand these factors, some researchers have made use of a classical measure, the taxonomic unit (e.g. species) [10,14] but it does not take into account ecological and evolutionary attributes of species. The use of taxonomic unit alone could provide an incomplete pattern of the man-made consequences on biodiversity [3]. However, some modern approaches are combining functional attributes of species (individual characteristics that can be measured and that affect the fitness) with phylogenetic relationships. This approaches, in addition to the taxonomic diversity, can bring different answers of a species community in relation to its habitat conditions, being these a combination of ecological and evolutionary answers [15,16]. Environmental degradation process can be observed by studying diversity measures that are affected by disturbance conditions [17], and for this, the usage of functional, phylogenetic and taxonomic diversity is a growing tool that have been changing the focus of researchers from the use of species diversity or species composition, that take no account of differences in species' life-history traits and ecological niches [17–19].

Although plenty of studies have shown the strong relationships between community structure and environmental predictors and how the functional traits of species can match up with the environmental conditions [20] some adaptive processes remain unclear, and this may be due the large number of traits presented by each species and/or the high species number existing in many habitats which generates an incomplete knowledge of which species traits can actually be an influence to ecosystems processes [21]. Among all vertebrates, the amphibians are the group with highest proportion of species threatened with extinction [22], due to habitat loss, fragmentation [23] and other related environmental stressors like enhance on UV radiation incidence [24,25] and canopy coverage loss [26]. Furthermore, the complexity on amphibian life cycle and the differences in life-history strategies between species and also their habitat associations generates a need for studies aiming to understand the true relationship between anthropogenic disturbance and the structure and organization of amphibian communities [7].

In the present study, we aimed to answer the following question: In relation to the anthropogenic disturbance in an agricultural- forest preserved gradient, ponds in more preserved environment would harbour higher taxonomic, functional and phylogenetic diversity patterns? So we tested the hypothesis that ponds located at most preserved and heterogeneous would be taxonomically richest and would allow the coexistence of more functionally distinct species [27], expecting then, a higher functional diversity and lower functional redundancy. We also expect an increase of the phylogenetic diversity (and thus a decrease in phylogenetic redundancy) at these sites, since more heterogeneous habitats can provide a wide range of microhabitat usage, diminishing the interspecific competition and allowing the coexistence of taxa with higher phylogenetic similarity [28,29].

2. Methods

2.1 Description of the study site

The sampling areas are located at the Parque Estadual do Turvo (PET) and its adjacencies, and both belongs to the Atlantic Rainforest biome. The PET is located at the Rio Grande do Sul State (27◦ 07’–27◦ 16’ S, 53◦ 48’–54◦ 04’ W; 100–400 a.s.l), at the municipality of Derrubadas, covering an area of 17,491 ha with about 90 km of perimeter of Semideciduous Forest. The study site differs from the wet evergreen forests since it is dryer and presents more open areas, sharing this same vegetational classification with the Republic of Argentina by the Moconá Provincial Park (about 1000 ha) and the Yabotí International Biosphere Reserve (236,613 ha), as well the Brazilian state of Santa Catarina by the Uruguay River [30] (Figure 1).

Vast majority of the surrounding areas of PET were converted into intensively agricultural landscapes dominated by crops of soybeans (~22.000 t/year) maize (7.560

t/year), wheat (6.840 t/year) and cattle (~8700 animals) pasture, and where the legal buffer zones are not implemented or respected [31]. The climate is characterized as subtropical highly-humid with average of rainfall between 1.700 and 1.900mm with reduction of precipitation at the winter season and the average of temperature ranges from 20ºC and 23ºC [32].

2.2 Data collection

The fieldwork was conducted during two anuran breeding seasons at southern Brazil, the first one from September (2013) to March of 2014 and the second from September (2015) to March of 2016, which comprises the spring and summer seasons at the southern hemisphere. The field campaigns were made monthly, during approximately 10 days, when we sampled 38 ponds following an environmental gradient ranging from an agricultural landscape to a preserved forest. This gradient ranged from the agricultural landscape where 19 ponds were located (outside of the park borders) to a preserved undisturbed forest, the inner portion of the gradient, with 19 ponds too (Figure 1). We made samples on adult anurans by using the method of "survey at breeding sites" [33], recording the number of calling males along all perimeter of ponds. Then, the maximum abundance data from each species in each pond, was used to construct the composition matrix to be used at the subsequent analysis.

We undertook a series of 13 measures (averages from the continuous values) on morphological and ecological traits from eight individuals of each recorded species, to access data on functional diversity and redundancy (Table 1). Since the sampling method is based on the calling males, the morphometric measures were taken only from adult males. The data acquisition was performed from anurans collected during the field campaigns and also on specimens already deposited at the Universidade Federal de Santa Maria collection (ZUFSM) between the years of 2010 and 2012 from the same area, to enlarge the database.

We also constructed a phylogenetic matrix based on the phylogenetic information of the species of [34] to access data on phylogenetic diversity and redundancy. We manually inserted six species (*Elachistocleis bicolor*, *Ololygon aromothyella*, *Physalaemus* aff. *gracilis*, *Rhinella ornata*, *Scinax granulatus* and *S. perereca*) not present at Pyron and Wiens work. The position of these insertions (missing species) was defined according the position of the closest species or closest species group [35–38].

2.3 Statistical analysis

We calculated the functional and phylogenetic patterns by using Rao´s quadratic entropy [39,40] and the taxonomic diversity by using the Gini-Simpson´s index, these analysis were based on Pillar et al. (2009) and Pillar and Duarte (2010) by constructing a dataset composed by four matrixes. The first one (matrix B, Appendix Table 1) containing the species functional traits, the second one (matrix W, Appendix Table 2) with the abundance of species in each sampled pond, the third one (matrix E, Appendix Table 3) with the environmental filter (distance from the nearest border of PET, negative values for outside and positive values for inside), and the fourth (matrix F, Appendix Table 4) with the phylogenetic information (transformed then into a matrix od phylogenetic distance) of the recorded species. To perform these analysis we used the software Phylocom [42] and SYNCSA (available at: http://ecoqua.ecologia.ufrgs.br/SYNCSA.html). As a way to explore our database and better understand the effects of richness and equability of the species distributed along the measured gradient of distance, we constructed a Whittaker's Diagram (or Dominance diagram). After these procedures, we tested the relation of the obtained values of functional diversity and redundancy, taxonomic diversity and redundancy and phylogenetic diversity and redundancy of each pond with its distance from PET's nearest border (positive values represented the ponds inside PET's area and negative values represented ponds outside PET's boundiers). In addition, we also tested the relation of the components of the community weighted means matrix (CWM matrix containing the weighted functional traits Appendix Table 5) with the distance from the PET's nearest borders (*Vegan* Package, *lm* function, R Development Core Team 2011).

3. Results

We found 15 anuran species from five families: Hylidae (five species), Leptodactylidae (four species), Bufonidae (two species), and Microhylidae and Ranidae, both with one species each. We registered all the 15 species in the inner portion of gradient (the portion inside the PET), and only 10 species in the outside portion. The most conspicuous species were *Dendropsophus minutus* and *Scinax granulatus* both occurring at 31 of the 38 sampled ponds respectively (Table 2). We found, based on the abundance distribution curve that the ponds located at inner portion of the gradient have the species abundance more equally distributed (equability) than the ponds located at the outside portion (Figure 2).

Regarding the taxonomic, functional and phylogenetic patterns of diversity that we analysed, only the phylogenetic diversity and phylogenetic redundancy were related to the studied gradient (r^2=0.14, p>0.05and r^2=0.20, p<0.05, respectively). The phylogenetic diversity (opposed to what we assumed) decreased at the inner portion of the gradient, while the phylogenetic redundancy increased (see Figure 3 A and B).

Despite the total functional diversity not show statistical significance, when evaluated alone with the distance gradient, the attributes (CWM matrix) showed that the individuals from the inner portion of the gradient, presented higher values of the forelimb ratios (hind limbs r^2=0.13, p<0.05, forearms r^2=0.11, p<0.05), and mouth size ratio (r^2=0.12, p<0.05) than the individuals found at the outside portion of the gradient. The results also showed that the reproductive mode number 4 [44], exhibited by *Hypsiboas faber* is more commonly found at the outside than the inner portion (r^2=0.12, p<0.05 see Figure 4 A-D).

The phylogeny generated concerning the anurans showed the formation of two distinct clades (Figure 5). The first, the oldest clade (about 90 million years) is formed by two genera with one species each (*Lithobates catesbeianus* and *Elachistocleis bicolor*), the second clade is formed by eight genera (*Physalaemus* [two species], *Leptodactylus* [two species], *Rhinella* [two species], *Phyllomedusa* [one species], *Dendropsophus* [one species], *Scinax* [three species], *Ololygon* [one species] and *Hypsiboas* [one species]).

4. Discussion

The anuran community of the PET is characterized as a mixture of species [45] given their distribution patterns. From the 15 species found at our study, five occurred only at the inner portion of the gradient (*Ololygon aromothyella, Phyllomedusa tetraploidea, Rhinella ornata, Scinax fuscovarius* and *Scinax perereca*), and four of them are quite dependent of the arboreal strata or the different types of vegetation at water surface (*O. aromothyella, P. tetraploidea, S. fuscovarius* and *S. perereca*) [44,46-47].

The pattern observed at the Whittaker’s diagram showed the dominance of a low number of species at the inside and outside portions of the gradient. This kind of pattern is considerably recurrent, other studies already showed the decrease of richness and enhancing on dominance at places affected by anthropogenic disturbance [52–54]. In the present case, at both portions of the studied gradient the native species *Dendropsophus minutus*, *Scinax granulatus* and the exotic species *Lithobates catesbeianus* presented higher abundances when compared to the other species. These two native and abundant species share not just the reproductive mode, but are also highly tolerant to human induced disturbances, being found close to human dwelling (or inside of them, like *Scinax* *granulatus*) and man-made waterbodies. However, *L. catesbeianus* presents a high invasive potential, and as explained by Madalozzo et al. [55] its distribution is facilitated by the influence of the edge effect and the man-made waterbodies along the borders of PET.

Our results show that despite the initial assumption of a higher taxonomic and functional diversity at the inner portions of the gradient, there is no significant difference between them. The great number of man-made waterbodies available outside of PET area may explain the similarity on the taxonomic and functional diversity given the high number of generalist species that inhabit both agriculture and forest environments (e.g. *Dendropsophus minutus, Scinax granulatus, Lithobates catesbeianus,* and *Physalaemus* spp.) and their reproductive modes, associated to both permanent and temporary ponds. This pattern of occurrence is commonly found at studies on Atlantic Rainforest *lato sensu* (with exception of the wet evergreen forest), mainly at locations that presents ecotonal characteristics (given the recent anthropogenic modifications). This landscape feature may exert influence on anuran reproductive behaviour and physiology, given the unpredictability of variables like temperature and evaporation at these places, enhancing the establishment of more plastic species which can respond differently and maybe more efficiently to disturbed environmental conditions [56,57]. In this way, it is expected to find similar species of the functional point of view when thinking only on the pond dwelling anurans, both, in and outside of the gradient, since they have to deal with the diversity of microhabitat of both places, diminishing the difference of this diversity pattern. However, when adding the stream (e.g. *Vitreorana uranoscopa*, *Hypsiboas curupi*, *Crossodactylus schmidti*) the marsh dwelling anurans (e.g. *Odontophrynus americanus*, *Proceratophrys avelinoi*, and *P. bigibosa*) and the extremely ephemeral pond dwelling anurans (e.g. *Melanophryniscus*) we can expect to see greater differences.

Despite the lack of difference on taxonomic and functional diversities (and also functional redundancy), we found evidence of a decrease on the phylogenetic component of diversity towards the interior of the park. This result suggests that the environmental filter (distance) have influence on the phylogenetic structure of the assemblages and also suggests low phylogenetic competition, opposing to what can be seen outside of the park [58,59]. However, another possibility may arise (concerning the decrease of phylogenetic diversity) with the presence of strong competitors (clades), in this case the competition would be also a biotic influence on these assemblages [60,61]. These two non-excluding possibilities agree with the hypothesis of the niche conservatism [61,62], so the similar ecological traits shared by the phylogenetically close species would allow them to coexist and the conservative similarity on niche usage by these species would have shaped the actual clustering or overdispersion (e.g. outside portion of the gradient).

In this way, it is expected to species that occupies the same habitat (e.g. inner portion or outside portion of the gradient) to show similar morphological traits in response to the environment [63]. However, under a more competitive scenario, it would be expected them to show differences on morphology, and then, showing the existence of some degree of niche specialization [64]. Despite the assumption of a similar response in morphology trait from closely related species (evolutionary), the difference found on the size of some morphological traits (e.g. hindlimbs, forearms and mouth), greater from individuals from inside and smaller to individuals from outside, here, this pattern occurs following the premises of adaptive radiation, showing that when the species (or lineages) adapt themselves to explore new or different niches, the changes can be rapid [65–67]. The individuals present at the inner portion of the gradient are adapted to cope with some barriers of dispersion (e.g. fallen trees, streams), and/or make use of a larger number of habitats than the individuals present on the outside portion that are susceptible to predation, dissecation and to pesticides (given the anthropic nature of the landscape). This is also corroborated when we see that species that construct nests that can hold water (reproductive mode 4) are better distributed or more frequently found at places with hydrological deficit.

It is widely known that land-use intensification is one of the major threats to biodiversity in local and global perspectives. Several studies showed that anthropogenic influence can cause a decline in several aspects of diversity in natural communities. In this way, these modifications would not allow the species to track their optimum environment, forcing them to adapt *in situ* to avoid extinction [68]. These adaptations can be seen when the functional traits (functional diversity) from individuals of a highly-preserved area, show no differences (or similarity) from individuals of a highly-converted area, it is, the phenotypical plasticity of these individuals is needed at these places. In the present study, we found evidence of a strong influence of the environmental conditions shaping the communities, given the phylogenetic clustering and the lack of difference on functional diversity.

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Tables

Table 1. Description of the ecomorphological traits of the anurans recorded in ponds monitored at Parque Estadual do Turvo and adjacencies between 2014 and 2016 *Reproductive modes based on

Trait type	Variable	Trait
Snout vent length	Continuous	Total size of the body, from the tip of the nose to the cloaca
Mouth ratio	Continuous	Distance between the rear edge of the jaw joint and the tip of the snout, divided by the snout vent length
Forelimb ratio	Continuous	Greater distance from the "shoulder" to the tip of the 'hand', divided by the snout vent length
Hind limb ratio	Continuous	Distance between the cloaca and the tip of the "foot", divided by the snout vent length
Perched	Binary	Place of activity/or vocalization
Ground	Binary	Place of activity/or vocalization
Water	Binary	Place of activity/or vocalization
Prolonged breeding	Binary	Time of breeding season
Explosive breeding	Binary	Time of breeding season
Reproductive Mode 1**	Binary	Reproductive mode
Reproductive Mode 2**	Binary	Reproductive mode
Reproductive Mode 11**	Binary	Reproductive mode
Reproductive Mode 24**	Binary	Reproductive mode
Reproductive Mode 30**	Binary	Reproductive mode

[44].

Table 2. Anuran species, place of occurrence, frequency of occurrence and maximum abundance recorded in ponds monitored at Parque Estadual do Turvo and adjacencies between 2014 and 2016.

Family/Species	Inside	Outside	Number of ponds (total 38)
Bufonidae			
Rhinella icterica	01	04	04
Rhinella ornata	34	00	07
Hylidae			
Dendropsophus minutus	74	40	31
Hypsiboas faber	30	24	18
Ololygon aromothyella	17	00	03
Scinax fuscovarius	04	00	01
Scinax granulatus	58	23	31
Scinax perereca	24	00	10
Leptodactylidae			
Leptodactylus latrans	27	13	12
Leptodactylus mystacinus	28	19	21
Physalaemus cuvieri	45	37	25
Physalaemus aff. *gracilis*	31	04	15
Microhilidae			
Elachistocleis bicolor	12	24	15
Phyllomedusidae			
Phyllomedusa tetraploidea	18	00	08
Ranidae			
*Lithobates catesbeianus**	50	28	23

*exotic species

Figures

Figure 1.

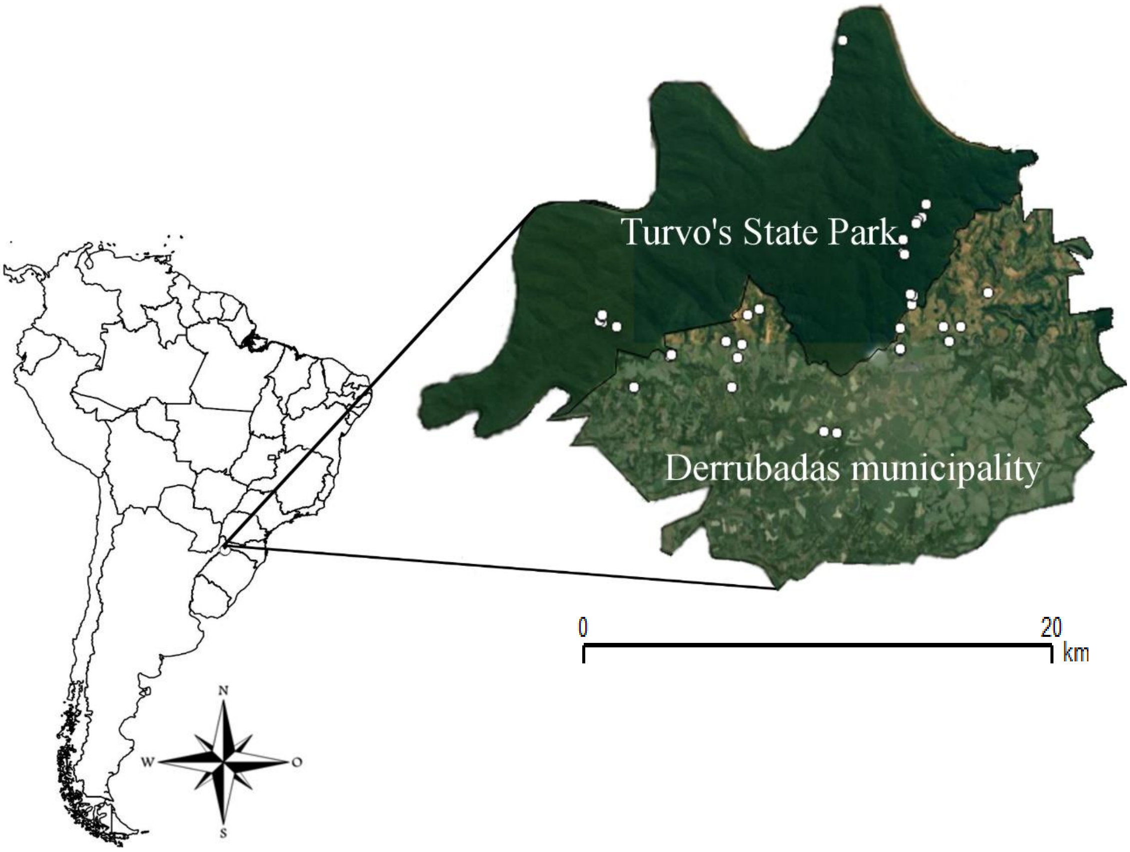

Figure 1. Map representing the sampling area on the extreme north-western of the Rio Grande do Sul State, Brazil. The white points on the map shows location of the sampled ponds along the agricultural-preserved forest gradient at Parque Estadual do Turvo and surrounds. The study was performed at two consecutive breeding seasons of anurans between 2014 and 2016.

Figure 2.

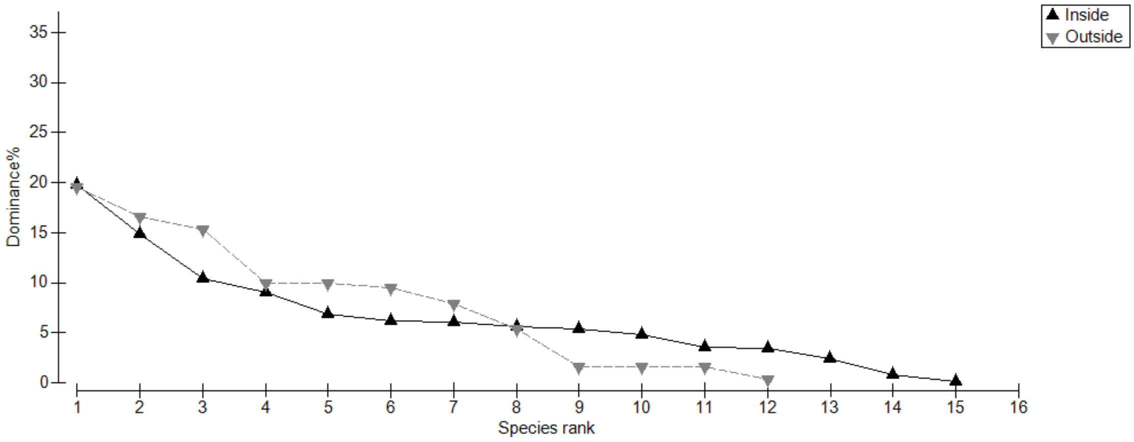

Figure 2. Whittaker diagram, showing the distribution of abundance of 15 anuran species recorded along an agricultural- preserved forest gradient at the Parque Estadual do Turvo, Rio Grande do Sul, southern Brazil. Black triangles represent the inner portion of the gradient, and the grey triangles represent the outside portion.

Figure 3.

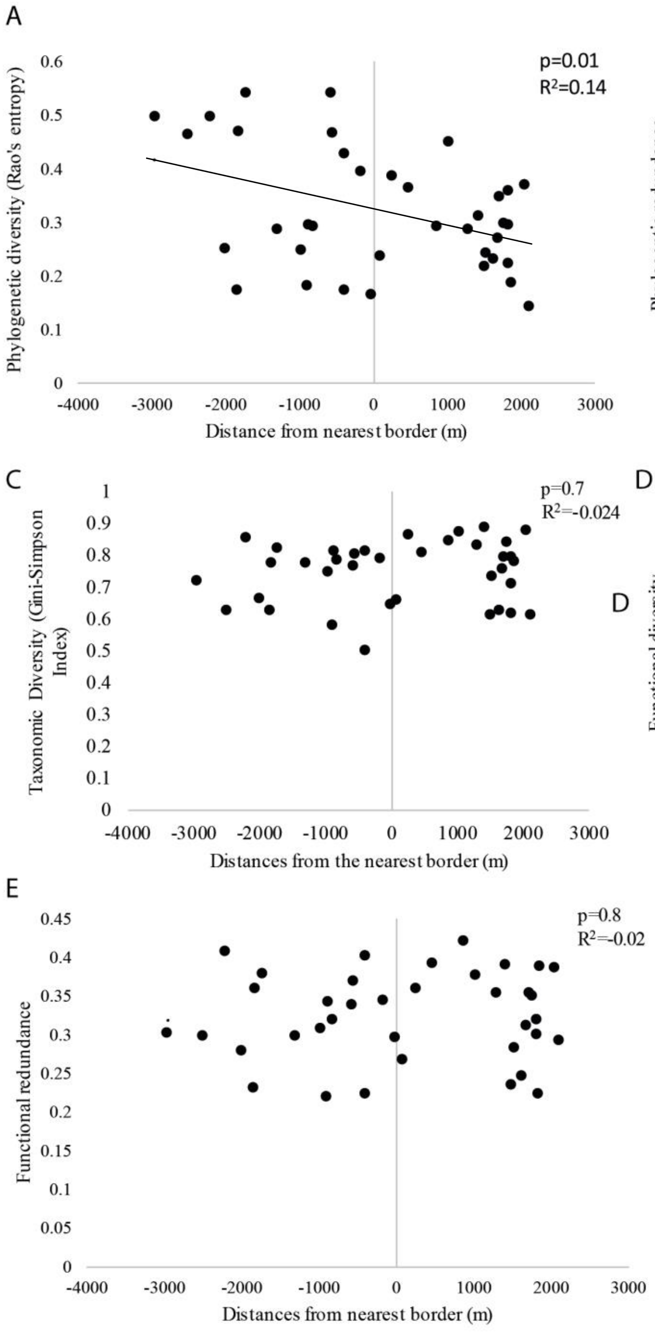

Figure 3. Regression results between anuran functional, phylogenetic ($p<0.05$) and taxonomic diversities and redundancy along the agricultural-preserved forest gradient (environmental filter) at Parque Estadual do Turvo and adjacencies, southern Brazil between 2014 and 2016.

Figure 4.

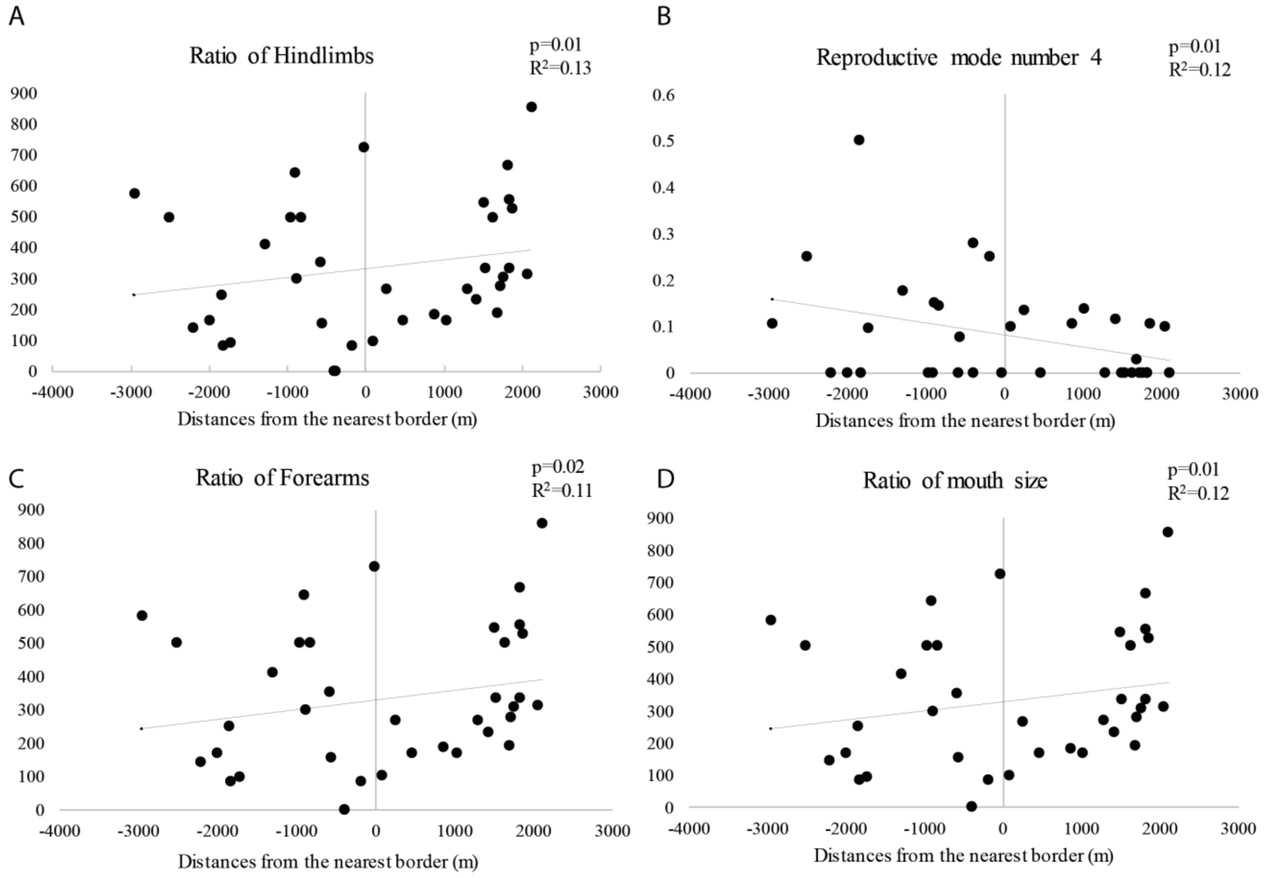

Figure 4. Regression results between the ratios of the morphological characteristics of anurans and their reproductive modes along the agricultural-preserved forest gradient (environmental filter) at Parque Estadual do Turvo and adjacencies, southern Brazil between 2014 and 2016.

Figure 5.

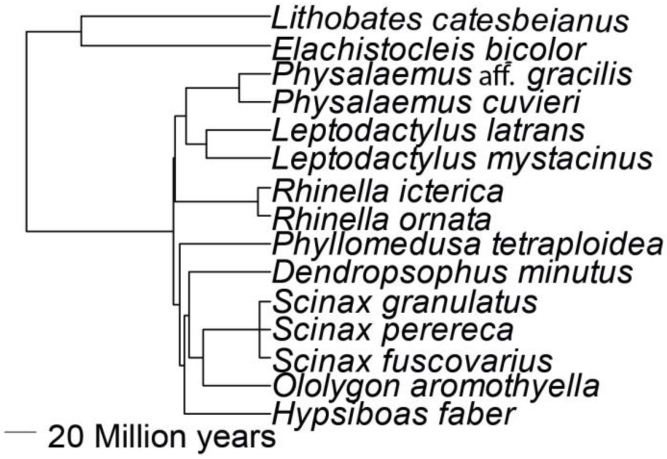

Figure 5. Phylogeny of the anurans recorded at waterbodies monitored along an environmental gradient ranging from an agricultural landscape to an well preserved forest at the southern Brazil. Generated based in Pyron and Wiens (2011), Narvaes and Rodrigues (2009), Faivovich (2002), Nascimento et al. (2005), de Sá et al. (2012, 2014), Vieira (2010). We defined the branche lenght based on the estimative of the age of the clades, given by the TimeTree (Hedges et al, 2006).

APPENDIX I- Examined anuran specimens from the Herpetological Collection of Universidade Federal de Santa Maria (ZUFSM). Missing numbers are individuals measured in field and from didactic collection.

Dendropsophus minutus*:* ZUFSM4540, ZUFSM4558, ZUFSM4621, ZUFSM4622, ZUFSM4630, ZUFSM4632; ***Elachistocleis bicolor:*** ZUFSM4575; ***Hypsiboas faber****:* ZUFSM *4476*, ZUFSM*4619*, ZUFSM*4661;* ***Leptodactylus fuscus****:* ZUFSM*4585*, ZUFSM*4628*, ZUFSM*4660*; ***Leptodactylus latrans***: ZUFSM4557, ZUFSM4604; ***Leptodactylus mystacinus***: ZUFSM4473, ZUFSM4526, ZUFSM4551;***Ololygon aromothyella:*** *ZUFSM4547, ZUFSM4566, ZUFSM4596, ZUFSM4598, ZUFSM4616, ZUFSM4623, ZUFSM4633, ZUFSM4634 ZUFSM4635*; ***Phyllomedusa tetraploidea****:* ZUFSM*4533*, ZUFSM*4580*, ZUFSM*4581;****Physalaemus cuvieri****:* ZUFSM*4555*, ZUFSM*4563*, ZUFSM*4578*, ZUFSM*4579;* ***Physalaemus*** **aff.** ***gracilis****: ZUFSM4356, ZUFSM4358, ZUFSM4359, ZUFSM4368, ZUFSM4553, ZUFSM4572, ZUFSM4609;* ***Rhinella icterica***: ZUFSM4529, ZUFSM4518, ZUFSM4516, ZUFSM4515, ZUFSM10000, ZUFSM10009, ZUFSM10010, ZUFSM10011; ***Rhinella ornata****:* ZUFSM4477, ZUFSM4496, ZUFSM4497, ZUFSM4498, ZUFSM4499, ZUFSM4527, ZUFSM4659, ZUFSM10005, ZUFSM10006, ZUFSM10007; ***Scinax fuscovarius****:* ZUFSM4549, ZUFSM4556, ZUFSM4576, ZUFSM4610; ***Scinax granulatus****:* ZUFSM4550, ZUFSM4559, ZUFSM4594, ZUFSM4607; ***Scinax perereca****:* ZUFSM2810, ZUFSM2956, ZUFSM4513, ZUFSM4597, ZUFSM4599, ZUFSM4606, ZUFSM4613, ZUFSM4617, ZUFSM4627, ZUFSM4808.

CONCLUSÃO

- O aumento, ainda que pequeno na incidência da radiação ultravioleta tem efeito extremamente negativo sobre larvas de *Hypsiboas curupi*, aumentando taxas de mortalidade, bem como de alterações morfológicas. Esses resultados ajudam a explicar a alta dependência dessa espécie quanto ao ambiente florestal e consequente desaparecimento em ambientes desmatados. É esperado que outras espécies de anuros, com ecologia e requerimentos de habitat similares, apresentem os mesmos padrões de resposta quanto ao aumento da radiação solar.
- As comunidades de anuros estudadas no Parque Estadual do Turvo e nas áreas agrícolas adjacentes não estão organizadas ao acaso. Essas comunidades estão estruturadas ao longo de um gradiente ambiental onde o processo de *turnover* é mais importante que o processo de aninhamento das comunidades. Ou seja, ocorre substituição das espécies de anuros à medida que os sítios de reprodução se distanciam dos ambientes mais antropizados.
- Para os estágios larvais dos anuros, existe uma variação na diversidade funcional entre as poças estudadas e um aumento neste componente à medida que as poças se distanciam da borda para o interior do Parque. No entanto, apesar da alta diversidade conhecida para o local, a formação de poucos grupos funcionais (sete) é preocupante, visto que a perda de um ou mais componentes destes grupos poderá acarretar em perdas significativas nos serviços ecossistêmicos prestados.
- No que tange a comunidade de anuros adultos do Parque Estadual do Turvo e das áreas convertidas associadas, vimos que não há diferença nos traços morfológicos das espécies que estão dentro para as que estão fora do Parque. Mostramos também que existe uma forte influência do ambiente regulando a diversidade

funcional das espécies e que a conversão da floresta nativa em sistema agrícola tende a aumentar a diversidade filogenética dos anuros nesta região.

ANEXOS

ANEXO 1