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ROSELI COELHO DOS SANTOS

INFLUÊNCIA DE FATORES AMBIENTAIS SOBRE A COMPOSIÇÃO, DIVERSIDADE FUNCIONAL E DOENÇAS INFECCIOSAS EM COMUNIDADES DE GIRINOS NO SUL DO BRASIL

SÃO LEOPOLDO
2022

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

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Na natureza nada se cria, nada se perde, tudo se transforma.

Antoine Lavoisier

RESUMO

Modificações na paisagem impactam a biodiversidade, alterando a composição de espécies presentes nas comunidades e colocando em risco de extinção as espécies mais vulneráveis. Os anfíbios são altamente susceptíveis às modificações ambientais, sendo afetados negativamente pela perda e fragmentação de habitat, mudanças climáticas, espécies exóticas e doenças. Devido a isso, os anfíbios são considerados o grupo mais ameaçado do planeta, com diversas espécies extintas ou em risco de extinção. No Brasil, o maior número de espécies ameaçadas se encontra na Mata Atlântica. Portanto, conhecer a diversidade taxonômica, atributos de diversidade funcional de girinos e a dinâmica de infecção causadas por patógenos, como a quitridiomicose e a ranavirose, é de extrema importância para a conservação dos anfíbios neste bioma. Na região sul do Brasil, remanescentes de Mata Atlântica formam um mosaico com áreas de pastagens, agricultura e urbanização. Estudos sobre ecologia de comunidades de girinos são escassos nesta porção da Mata Atlântica. Aqui, nós apresentamos estudos inéditos sobre a composição de comunidades de anuros, atributos de diversidade funcional e infecção por patógenos de doenças emergentes (quitridiomicose e ranavirose), usando larvas de anuros como modelos de estudo. Realizamos a coleta de dados em campo, entre outubro de 2018 a março de 2019, em corpos d’água (riachos e poças) localizados em remanescentes florestais na região sul do Brasil. Amostramos sete remanescentes florestais, nos estados do Paraná, Santa Catarina e Rio Grande do Sul. Coletamos aproximadamente 3000 girinos, de 22 espécies em 30 corpos d’água. Em laboratório, identificamos os girinos, realizamos medidas morfológicas e determinamos a presença de despigmentação no aparato bucal e zoosporângios, associados ao fungo *Batrachochytrium dendrobatidis* (*Bd*). Também utilizamos qPCR para identificar ranavirus nas comunidades estudadas. Utilizamos imagens de satélites para acessar o uso do solo das áreas amostradas, analisamos dados morfológicos dos microambientes e parâmetros físicos e químicos da água dos corpos d'água. Nossos resultados indicaram que a composição de comunidade de girinos foi influenciada por maiores áreas de florestas e pastagens. Os atributos de diversidade funcional dos girinos foram selecionados por características físico-químicas da água e características morfológica do habitat, atuando de forma distinta entre os corpos d'água lênticos e lóticos. Não registramos a presença de ranavirus nas comunidades estudadas, porém, encontramos elevadas prevalências de *Bd*. As comunidades de girinos inseridas em paisagens com maiores áreas de florestas foram indicadoras de *Bd*, assim como duas espécies especialistas de habitat (*Boana curupi* e *Crossodactylus schmidti*) e uma generalista de habitat (*Boana faber*), mas que utiliza áreas florestais para abrigo. Assim, concluímos que o sul da Mata Atlântica apresenta elevadas prevalências de *Bd* e os ambientes florestais exercem maior influência sobre a dinâmica deste fungo nos ambientes aquáticos. Nosso estudo fornece dados importantes sobre a diversidade taxonômica, atributos de diversidade funcional e vulnerabilidade a patógenos emergentes em comunidades de anuros em uma região pouco conhecida da Mata Atlântica.

Palavras-chave: Atributos de diversidade funcional. Composição de espécies. Larvas de anuros. Mata Atlântica. Quitridiomicose.

ABSTRACT

Landscape changes impact biodiversity, altering the species composition of a community and threatening the more vulnerable species with extinction. Amphibians are highly susceptible to environmental changes and are negatively affected by habitat fragmentation and loss, climatic changes, exotic species, and diseases. Due to this, amphibians are considered the most threatened group on the planet, with several species already extinct or threatened with extinction. The largest number of threatened species in Brazil occur in the Atlantic Forest. Therefore, knowing the composition, functional diversity attributes, and dynamics of the infection caused by pathogens such as chytridiomycosis and ranavirosis is extremely important for amphibian conservation in this biome. In Brazil's South Region, remnants of the Atlantic Forest form a mosaic with areas of pastures, agriculture and urbanization. Studies on the ecology of tadpole communities are scarce in this portion of the Atlantic Forest. Here, we present unprecedented studies on anuran community composition, functional diversity attributes, and infection by pathogens of emergent diseases (chytridiomycosis and ranavirosis) using tadpoles as study models. We conducted field collections between October 2018 and March 2019 in waterbodies (streams and ponds) located in forest remnants in Brazil's South Region. The study area included seven forest remnants in the states of Paraná, Santa Catarina, and Rio Grande do Sul. We collected about 3000 tadpoles from 22 species in 30 waterbodies. In the laboratory, we identified the tadpoles and determined the presence of deformities in the oral apparatus and zoosporangia of the fungus *Batrachochytrium dendrobatidis* (*Bd*) and performed morphological measurements. We also used qPCR to identify ranavirus in the studied communities. We used satellite images to assess land use of the sampled areas, analyzed morphological data of the microenvironments and physical and chemical parameters of the water in the waterbodies. We showed that the dissimilarity in the composition of tadpole communities was influenced by larger areas of forest and pasture, with the habitat-specialist species being associated with forests. Attributes of tadpole functional diversity were selected by physicochemical characteristics of the water and morphological characteristics of the habitat, which act differently on lentic and lotic waterbodies. We did not record the presence of ranavirus in the studied communities but found a high prevalence of *Bd*. Tadpole communities in landscapes with larger forest areas were indicators of the *Bd* fungus. Similarly, two habitat-specialist species (*Boana curupi* and *Crossodactylus schmidti*) and one habitat-generalist (*Boana faber*) were *Bd*-

indicator species, the latter using forest areas as shelter. Thus, we conclude that the southern Atlantic Forest has a high prevalence of *Bd*, and forest environments have a greater influence on the dynamics of this fungus in the aquatic environments. Our study provides important data on the composition, functional diversity, and vulnerability to emergent pathogens in anuran communities in a little-known region of the Atlantic Forest.

Keywords: Functional-diversity attributes. Species composition. Anuran larvae. Atlantic Forest. Chytridiomycosis.

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SUMÁRIO

APRESENTAÇÃO

Esta tese está estruturada em quatro capítulos, os quais abordam temáticas referentes à diversidade e ameaças à anurofauna da região sul da Mata Atlântica. O primeiro capítulo é constituído pelo referencial teórico, organizado de forma a salientar a importância dos estudos com ecologia de comunidades de anfíbios, a influência do ambiente sobre a formação das comunidades e os atributos de diversidade funcional dos girinos. Além disso, apresentamos uma caracterização e histórico sobre as doenças infecciosas emergentes (quitridiomicose e ranavirose), que acometem os anfíbios e estão ligadas a declínios e extinções em todo o mundo nas últimas décadas.

O segundo capítulo aborda a influência da paisagem na composição de comunidades de anfíbios. Este capítulo foi publicado na revista Herpetological Conservation Biology (http://www.herpconbio.org/Volume_16/Issue_3/Santos_etal_2021.pdf), com fator de Impacto 1,13, estando, portanto, formatado nas normas desta revista.

O terceiro capítulo aborda o tema de diversidade funcional com comunidades de girinos, analisando a associação atributo-ambiente, das características morfológicas dos girinos em resposta aos descritores ambientais. Este capítulo está formatado nas normas da revista Aquatic Ecology, Fator de Impacto 1,64, para a qual está em fase de revisão.

O quarto capítulo aborda sobre a incidência de doenças infecciosas (quitridiomicose e ranavirose) nas comunidades de girinos e sua associação com o uso de solo. Este capítulo está formatado nas normas da revista Ecological Indicators, Fator de Impacto 4,95, para a qual está sendo preparada a submissão.

CAPÍTULO 1 – Referencial teórico – Composição, diversidade funcional e doenças infecciosas em comunidades de girinos

A Mata Atlântica e a fragmentação de habitats

A Mata Atlântica é um dos principais “hotspot” de biodiversidade mundial (MYERS, 2000; MITTERMEIER *et al*., 2011), com 298 espécies de mamíferos (ABREU *et al*., 2021), cerca de 600 espécies de anfíbios (ROSSA-FERES *et al*., 2017), mais de 300 espécies de répteis, 1.000 espécies de aves e 23.000 espécies de plantas (HANCE e BUTLER, 2022), apresentando elevado grau de endemismo. Devido ao intenso processo de ocupação e exploração experienciado ao longo dos últimos séculos, esse bioma passou a ser considerado um dos mais ameaçados mundialmente, em termos de biodiversidade (RIBEIRO *et al*., 2009; FUNDAÇÃO SOS MATA ATLÂNTICA, 2019). No Brasil, a Mata Atlântica ocupa mais de 1.540.000 km² e equivale a cerca de 17% do território (JOLY *et al*., 2014). Porém, restam apenas 12,4% da sua área original (FUNDAÇÃO SOS MATA ATLÂNTICA, 2019). Existem atualmente cerca de 245.000 fragmentos florestais, sendo que os maiores remanescentes estão em áreas mais íngremes e de difícil acesso, especialmente ao longo das serras litorâneas (SILVA *et al*., 2007). Em altitudes menores (entre 400 e 800 m), estão as áreas mais fragmentadas do bioma (RIBEIRO *et al*., 2011). A Mata Atlântica também concentra a maior proporção (cerca de 70%) da população humana no Brasil, o que contribui para a intensificação da conversão de áreas de florestas em cultivos agrícolas, pastagens e habitações urbanas (RIBEIRO *et al*., 2009). A Mata Atlântica original apresenta enorme heterogeneidade de fitofisionomias, das quais destacam-se cinco principais tipos de florestas: Floresta Ombrófila Densa, Floresta Ombrófila Aberta, Floresta Ombrófila Mista, Floresta Semidecidual Sazonal e Floresta Decidual Sazonal (JOLY *et al*., 1999; OLIVEIRA FILHO e FONTES, 2000). No sul do Brasil há predomínio de Floresta Ombrófila Densa e Floresta Ombrófila Mista ao longo dos gradientes altitudinais (OLIVEIRA FILHO e FONTES, 2000), associadas com outras fitofisionomias, como os campos naturais (IAP, 2004). A redução das florestas implica na também redução da biomassa das plantas e alteração dos processos sucessionais da flora (JOLY *et al*., 2014), acentuando a homogeneização da biota. Em algumas regiões, a paisagem pode ser representada como um arquipélago de pequenas ilhas de vegetação natural, cercadas por uma matriz de áreas degradadas com pastagens, agricultura, silvicultura e áreas urbanas (JOLY *et al*., 2014; REZENDE *et al*., 2018). Esse processo gera efeitos negativos diretos à biota (TSCHARNTKE *et al*., 2005; TABARELLI *et al*., 2010), ao reduzir a complexidade e a heterogeneidade de habitat (MACHADO *et al*., 2012; SACCOL *et al*., 2017), e consequentemente, a riqueza de espécies (BARROWS e ALLEN, 2010). Quando o habitat natural é substituído, muitas interações ecológicas são afetadas, como predação, competição e relações hospedeiros/parasitas (NOMURA *et al*., 2011; LAUFER *et al*., 2015; PREUSS *et al*., 2020; SANTOS *et al*., 2020). Essas alterações podem variar de acordo com a intensidade da degradação (RILEY *et al*., 2005; BRAND *et al*., 2010).

A região sul do Brasil vivenciou um acelerado processo de modificação na sua paisagem, sendo algumas fitofisionomias florestais, como a Floresta Ombrófila Mista, chegando a menos de 3% da sua área original (GALINDO-LEAL e CÂMARA, 2003). As principais atividades estão relacionadas à agropecuária (RIBEIRO *et al*., 2011), os poucos remanescentes florestais existentes estão incluídos em Unidades de Conservação (UCs) e a maioria são formados por reservas legais e corredores ecológicos (REZENDE *et al*., 2018). Um dos efeitos negativos da agricultura é a contaminação por pesticidas e herbicidas (KOUMARIS e FAHRIG, 2016), favorecendo para a redução gradual na diversidade de espécies (MCKINNEY, 2008; BARROWS e ALLEN, 2010). À medida que a ocupação humana de uma paisagem aumenta, a proximidade entre habitats naturais e áreas urbanas também aumenta, causando uma série de impactos indiretos (e menos estudados) sobre a fauna, como poluição luminosa (DIAS *et al*., 2019), poluição sonora (PELLET *et al*., 2004) e atropelamentos (DINIZ e BRITO, 2015). Esses efeitos destacam a relevância do monitoramento contínuo das mudanças na paisagem induzidas pela ocupação humana sobre a biodiversidade.

A avaliação da paisagem é uma importante ferramenta para acessar informações sobre os ecossistemas, auxiliando na análise dos riscos para a manutenção e conservação da biodiversidade e, consequentemente, subsidiar a gestão ambiental. Em nível de espécie, a configuração da paisagem afeta a persistência da fauna (FAHRIG e NUTTLE, 2005), que é uma linha de base para testar modelos de metapopulações e metacomunidades. Em geral, as espécies com baixa capacidade de dispersão, como anfíbios, são mais sensíveis às mudanças da paisagem (SCHMUTZER *et al*., 2008; DIXO e METZGER, 2010; DINIZ e BRITO, 2013; CAYUELA *et al*., 2015). A maioria dos anfíbios tem parte de seu ciclo reprodutivo dependente de habitats aquáticos, e mudanças nas paisagens podem restringir o acesso a lagoas ou riachos (BECKER *et al*., 2007, 2010; MACHADO *et al*., 2012) formando barreiras geográficas (CAYUELA *et al*., 2015) que podem levar a uma modificação na estrutura taxonômica, funcional e filogenética das comunidades (DALMOLIN *et al*., 2019). Isso pode reduzir o sucesso reprodutivo, causando mudanças na estrutura das comunidades (BERRIOZABAL-ISLAS *et al*., 2018; DALMOLIN *et al*., 2020), declínios populacionais e extinções locais

(MARSH e TRENHAM, 2001; ROTHERMEL, 2004; GOUTTE *et al*., 2013). Portanto, os anfíbios são considerados bons modelos para estudos ecológicos na compreensão do impacto das mudanças da paisagem na vida selvagem. O monitoramento das populações de anfíbios tem preenchido lacunas e sobre como as mudanças na paisagem afetam a diversidade de espécies (BECKER *et al*., 2007; PILLSBURY e MILLER, 2008; NOMURA *et al*., 2011; COLLINS e FAHRIG, 2017).

Diversos estudos avaliam a relação entre a diversidade de espécies de anuros e a heterogeneidade de habitat (GONÇALVES *et al*., 2015; KNAUTH *et al*., 2018; FIGUEIREDO *et al*., 2019). Todavia, ainda existem limitações quanto às avaliações em escalas espaciais mais amplas, que considerem, por exemplo, a paisagem do entorno dos habitats. As análises da paisagem permitem a detecção de mudanças no uso e ocupação do solo em escalas compatíveis com as espécies ou grupos em questão (HAMER e PARRIS, 2011), ajudando a definir estratégias de conservação locais e regionais.

Diversidade funcional

Diversidade é um termo usado comumente como índice de medida de riqueza e abundância de espécies (WHITTAKER, 1972; LANDE, 1996; PURVIS e HECTOR, 2000), e suas aplicações visam entender a sua relação com o funcionamento dos ecossistemas. Seu conceito envolve variações entre as espécies, dentro de seus genótipos e fenótipos, além de variações espaciais e temporais nas comunidades e ecossistemas (CALAÇA e GRELLE, 2016). A riqueza e a diversidade taxonômica foram por muito tempo os componentes mais utilizados em estudos ecológicos (GASTON, 1996; PURVIS e HECTOR, 2000; MAGURRAN, 2004). Porém, nas últimas décadas é notável o crescente número de estudos de ecologia incluindo uma outra dimensão da diversidade: a diversidade funcional (SCHLEUTER *et al*., 2010). A diversidade funcional é um dos componentes da biodiversidade e sua definição se refere ao valor da variação das espécies e características do organismo que influenciam o funcionamento do ecossistema (TILMAN, 2001). Em outras palavras, é a medida do valor e da variação das características funcionais prevalentes no ecossistema (GOSWAMI *et al*., 2017), levando em conta a complementaridade e a redundância na co-ocorrência de espécies (DÍAZ e CABIDO, 2001; PETCHEY e GASTON, 2006). A diversidade funcional leva em consideração as diferenças morfológicas, ecológicas e comportamentais entre as espécies e indivíduos (CALAÇA e GRELLE, 2016), comumente conhecidas por atributos funcionais. A diversidade funcional representa a extensão das diferenças entre espécies baseada na distinção de suas características morfológicas, fisiológicas e ecológicas (PETCHEY e GASTON, 2002). Já o atributo funcional é qualquer característica fenotípica mensurável nos indivíduos (e.g. morfológica, comportamental, fisiológica) que afete, de maneira direta ou indireta, seu desempenho em crescer, reproduzir e sobreviver (MCGILL *et al.*, 2006; VIOLLE *et al.*, 2007).

Uma das justificativas para o uso de medidas de atributos de diversidade funcional é o consenso de que as espécies contribuem de maneiras distintas para a diversidade da comunidade e para o funcionamento do ecossistema, e que a relação entre a diversidade e funcionamento pode ser mais bem explicada via atributos funcionais ao invés de simples medidas que não incorporam diferenças interespecíficas, como a riqueza de espécies (LAWTON, 1994; GRIME, 1998; DÍAZ e CABIDO, 2001). Assim, uma das maiores preocupações está voltada para a escolha da maneira mais adequada de se medir esse componente da biodiversidade (PODANI e SCHMERA, 2006; MOUCHET *et al.*, 2008; POOS *et al.*, 2009).

Para analisar o funcionamento de um ecossistema é de suma importância levar em conta o número de espécies, a diversidade e a abundância das medidas de atributos funcionais registrados dentro de uma comunidade (LAVOREL e GARNIER, 2002; DÍAZ *et al.*, 2007). Os atributos funcionais variam de acordo com o nível de organização dos seres vivos, sendo que para comunidades os atributos biológicos analisados ao nível de indivíduos refletem nos padrões de ocorrência e manutenção da espécie nos ambientes, considerando a sua habilidade de adaptar-se às condições ambientais locais, ou seja, que são mediados por filtros ecológicos (VIOLLE *et al.*, 2007).

Os filtros ecológicos podem ser definidos de acordo com sua escala espacial (WEIHER *et al.*, 2011; VIOLLE *et al.*, 2012). As escalas espaciais maiores, em nível de macroambientes, comportam uma alta variedade de habitats (WEBB *et al.*, 2002; SOBRAL e CIANCIARUSO, 2012). Nesta escala, os filtros são externos à comunidade, incluindo o tipo de clima, solo, relevo, presença e ação de predadores generalistas fazem a triagem das espécies potenciais de chegar na comunidade local, oriundos da comunidade regional (RICKLEFS, 1987, VIOLLE *et al.*, 2012). Distintamente, em escalas espaciais menores, os habitats tendem a ser mais homogêneos (WEBB *et al.*, 2002; SOBRAL e CIANCIARUSO, 2012), a interação entre os organismos regula a densidade e as características ambientais internas, que se somam a configuração do habitat e do micro-habitat (e.g. heterogeneidade) e atuam como filtros, regulando a persistência das espécies na comunidade local (VIOLLE *et al.*, 2012).

Além disso, uma comunidade ecológica é definida como um conjunto de organismos que ocupam uma mesma área geográfica, em um mesmo espaço e tempo (RICKLEFS, 1987), sendo definida pela natureza da interação entre as populações ou pelo local onde vivem

(RICKLEFS e MILLER, 1999). Sua formação e manutenção se dá pela entrada de espécies colonizadoras advindas de um banco regional (*pool*) de espécies (HILLERISLAMBERS *et al*., 2012; KRAFT *et al*., 2015). Assim, para que uma espécie possa se estabelecer em um ambiente, ela precisa apresentar um crescimento populacional positivo ao longo do tempo, definindo seu micro-habitat dentro do microambiente (CHESSON, 2000). Neste nível ambiental, ela passa a estabelecer relações intraespecíficas e interespecíficas, refletindo em alterações físicas no macroambiente (ERWIN, 2008). Pode-se considerar que a relação entre a espécie e sua adaptação ao microambiente se caracteriza pela construção do nicho e sua persistência facilita a entrada de outras espécies, promovendo alterações no macroambiente influenciada pelos filtros ambientais (THAKUR e WRIGHT, 2017). Assim, os filtros ambientais e/ou ecológicos (abióticos e bióticos) atuam na seleção natural influenciando as "regras de montagem" ao selecionarem espécies que possuem características similares, as quais as permitem sobreviver sob determinadas condições impostas pelo ambiente (CORNWELL *et al*., 2006; SOBRAL e CIANCIARUSO, 2012). Os filtros ambientais regulam desde a chegada até a persistência das espécies na comunidade local, seleciona as espécies de acordo com os seus atributos funcionais, num processo similar à seleção de genótipos (KEDDY, 1992; WEIHER e KEDDY, 1995; WEIHER *et al*., 2011).

Características de diversidade funcional dos girinos

Os anfíbios, em especial os girinos, apresentam elevada dependência da água (DUELLMAN e TRUEB, 1994; WELLS, 2007). Das aproximadamente 8.000 espécies de anuros descritas atualmente (AMPHIBIAWEB, 2021), a maioria apresenta fase larval (ALTIG e MCDIARMID, 1999) com desenvolvimento indireto, ou seja, os ovos são depositados na água, ocorre a eclosão dos girinos, que permanecem na água até completar a metamorfose, atingindo a fase adulta (HADDAD e PRADO, 2005). Principalmente nas regiões neotropicais, os girinos ocorrem em diversos habitats de água doce incluindo lagoas, riachos, fitotelmata de bromélias, troncos e lâminas d'água rasas permanentes e temporárias (ALTIG e MCDIARMID, 1999). Os girinos apresentam grande variação nas características ecológicas e morfo-funcionais influenciados por diferentes pressões seletivas do ambiente (DUELLMAN e TRUEB, 1994; ALTIG e MCDIARMID, 1999; ROELANTS *et al*., 2011; SHERRATT *et al*., 2017, 2018).

As principais variações nas características morfológicas estão relacionadas a forma do corpo, das nadadeiras e do aparato bucal (DUELLMAN e TRUEB, 1994; ALTIG e

MCDIARMID, 1999). A posição do disco oral reflete na ecologia trófica dos girinos, sendo que as variações mais comuns são relacionadas a sua posição no corpo: ventral, anteroventral e anterior (terminal) (ALTIG e JHONSTON, 1989). A configuração das papilas marginais da boca é importante para a identificação de espécies e sua classificação nas guildas tróficas, particularmente de espécies de riachos, que estão relacionadas à aquisição de alimentos (ALTIG e JHONSTON, 1989). O aparato bucal é composto pelas papilas marginais, bico córneo, fileira de dentes e estruturas bucofaríngeas. Embora, havendo uma variação nas configurações entre os organismos, todos convergem para a função alimentar, e tais variações permitem a formação das guildas alimentares dos girinos (ALTIG e JHONSTON, 1989). O formato do corpo, da cauda e a posição dos olhos estão relacionados com a exploração do habitat aquático, tais como deslocamento e posição na coluna d'água (ALTIG e JHONSTON, 1989). Associando características morfológicas externas com hábitos alimentares, padrões ontogenéticos e posição na coluna da água, Altig e Jhonston (1989) classificaram os girinos em 18 guildas ecomorfológicas. Desta forma, consideraram nectônicos, os girinos que apresentam formato do corpo comprimido lateralmente, olhos laterais, musculatura caudal menos robusta associada a nadadeiras altas, podendo apresentar flagelo (ex. Hylidae; Figura 1A), enquanto os girinos bentônicos, de forma geral, apresentam corpo deprimido dorsoventralmente, olhos dorsais ou dorsolaterais e nadadeiras baixas (ex. Leptodactylidae e alguns Hylidae; Figura 1B).

Nos girinos planctônicos (neustônicos) são incluídos os suspensos-filtradores que não possuem peças bucais queratinizadas, flagelo da cauda comum, corpo geralmente fortemente deprimido, olhos com posição lateral, nadadeiras da cauda baixas com tamanhos semelhantes ou ventrais maiores, podendo se movimentar na coluna d'água e suspensos-raspadores, apresentam peças bucais queratinizadas, com fileiras de dentes (geralmente com fórmula 2/3) e com interrupção nas papilas marginais, olhos posicionados lateralmente, flagelo da cauda presente em diferentes graus, ficam frequentemente suspensos no meio da água e de cabeça para cima, com nadadeiras da cauda ventral mais alta do que a dorsal, com junção do corpo-cauda (ex. Phyllomedusidae; Figura 1C). Além disso, alterações em estruturas orais, como aumento do número de fileiras de dentículos e presença de papilas marginais por toda extensão do disco oral que foram associadas a diferentes ambientes lóticos e lênticos, respectivamente (ALTIG e JHONSTON, 1989; MCDIARMID e ALTIG, 1999). Apesar desta classificação, a qual associa grande especificidade entre morfologia e modo de vida ou habitat, os girinos apresentam plasticidade fenotípica em resposta a diversas variáveis ambientais. Tal variação fenotípica pode responder a variações na intensidade do risco de predação e a densidade de girinos entre lagoas, ou mesmo em níveis espaciais, sendo destacadas variações até mesmo dentro de porções distintas de um mesmo habitat (MICHEL, 2011).

Figura 1. Vista lateral de girinos das guildas ecomorfológicas. Em (A), girino da guilda nectônica, espécie *Scinax fuscovarius*; (B) girino da guilda bentônica, espécie *Physalaemus cuvieri;* (C) girinos da guilda neustônica, espécie *Phyllomedusa tetraploidea*. Fonte: Elaborada pela autora com imagens adaptadas de GONÇALVES (2014).

Quitridiomicose

Além da paisagem influenciar na composição de espécies de anfíbios, as doenças pandêmicas como a quitridiomicose e a ranivirose também influenciam na sobrevivência destes organismos no ambiente. Uma das doenças pandêmica mais alarmantes é causada pelo fungo quitrídeo, *Batrachochytrium dendrobatidis* (*Bd*) (BERGER *et al*., 1998; LIPS, 1999; DASZAK *et al*., 2003; STUART *et al*., 2004), que atualmente vem promovendo declínios e extinções dos anfíbios em todo o mundo (STUART *et al*., 2004). A destruição e alteração de habitat associados ao comércio e a introdução de espécies exóticas, mudanças climáticas, contaminação química e patógenos são os principais fatores que contribuem para o aumento da quitridiomicose (WAKE e VREDENBURG, 2008; HAYES *et al*., 2010; LIU *et al*., 2013). Nos anos de 1990, os declínios de anfíbios passaram a ser associado ao fungo *Bd*, aumentando a relevância de compreender seu mecanismo de ação (BERGER *et al*., 1998; LIPS, 1999; DASZAK *et al*., 2003; STUART *et al*., 2004; O'HANLON *et al*., 2018). Sua ocorrência já foi registrada em todos os continentes, exceto na Antártida (O'HANLON *et al*., 2018), tendo infectado mais de 700 espécies de anfíbios, ocasionando eventos de mortalidade e extinções locais (LIPS, 2016). O surgimento e dispersão de linhagens virulentas por todo o planeta, é atribuída principalmente ao comércio e a introdução de espécies exóticas (LIU *et al*., 2013). A Ásia é apontada como um provável local de origem do *Bd*, com linhagens endêmicas (O'HANLON *et al*., 2018), porém, existem outras linhagens endêmicas, como o *Bd*-Brasil que coexiste com o *Bd*-GPL, uma linhagem panzoótica global (SCHLOEGEL *et al*., 2012), ocorre principalmente em regiões subtropicais (GREENSPAN *et al*., 2018). Estudos recentes mostram que híbridos formados a partir do *Bd*-Brasil e do *Bd*-GPL formam linhagens com maior potencial de virulência (GREENSPAN *et al*., 2018), podendo elevar os riscos de infecção dos anfíbios (OLSON e RONNENBERG, 2014).

Rápidos declínios e extinções ocorrem em diversas partes do mundo pela combinação de dois ou mais fatores (BEEBEE e GRFFITHS, 2005; CARNAVAL, 2006; WAKE e VREDENBURG, 2008), que atuam sinergicamente resultando em estresse fisiológico e imunossupressão nos anfíbios, facilitando a ocorrência de infecções por agentes patogênicos (SCHAR *et al*., 2004; HAYES *et al*., 2010). A ocorrência do fungo *Bd* está associada a fatores bióticos e abióticos como a temperatura (PIOTROWSKI *et al*., 2004; SONN *et al*., 2017), pH (PIOTROWSKI *et al*., 2004; SIMPKINS *et al*., 2017) e altitude (PIOVIA-SCOTT *et al*., 2011). Seu impacto depende da capacidade de resistência das espécies (BLAUSTEIN *et al*., 2012; KRYNAK *et al*., 2016). O *Bd* apresenta crescimento ótimo entre 17 e 23ºC, não tolerando temperaturas superiores a 29ºC e inferiores a 0ºC (PIOTROWSKI *et al*., 2004; LIPS *et al*., 2008; WOODHAMS *et al*., 2008).

Anfíbios que vivem em ambientes com temperatura entre ~10-16ºC têm mais risco de infecção do que outras espécies de clima quente (GREENBERG *et al*., 2017). E espécies com sistema de reprodução explosiva tem menor risco de infecção (GREENBERG *et al*., 2017). Desta forma, a quitridiomicose afeta diferentemente os anfíbios ao longo do ciclo de vida (RACHOWICZ e VREDENBURG, 2004; CATENAZZI *et al.*, 2013). Girinos normalmente apresentam elevadas prevalências e carga de infecção, porém, não foram reportados eventos de mortalidade, associando-os à condição de reservatório do *Bd* (GARNER *et al*., 2009).

Girinos são comumente utilizados em experimentos com exposição ao fungo *Bd* (GERVASI *et al*., 2013; MARTEL *et al.,* 2013). Por não apresentarem altos índices de mortalidade, os girinos são considerados como reservatórios da doença (DASZAK *et al*., 2003; RACHOWICZ e VREDENBURG, 2004; BLAUSTEIN *et al*., 2005; VALENCIA-AGUILAR *et al*., 2016). Girinos de ambientes aquáticos permanentes apresentaram maiores cargas de infecção por *Bd* do que girinos de ambientes temporários, porém, menor prevalência (RUGGERI *et al*., 2018), sugerindo haver diferença entre ambientes lóticos e lênticos. A história de vida das espécies pode ser importante para entender a dinâmica do fungo (RUGGERI *et al*., 2018) principalmente em regiões como a Mata Atlântica, que além de ser um "*hotspot*" de biodiversidade (MITTERMEIER *et al*., 2005) e centro de endemismo (HADDAD *et al.,* 2013), é também um local em que o quitrídeo está amplamente distribuído (CARVALHO *et al*., 2017; GREENSPAN *et al*., 2018). Considerando que anfíbios com desenvolvimento direto podem ser mais susceptíveis à infecção (MESQUITA *et al*., 2017), a alteração do habitat tende a favorecer o patógeno na relação patógeno-hospedeiro, uma vez que a fragmentação do habitat leva ao isolamento de populações de anfíbios e facilita a disseminação do fungo (GREENSPAN *et al*., 2018). Neste contexto, não é possível compreender se tanto ambientes preservados quanto ambientes alterados apresentam as mesmas condições de prevalência e intensidade de infecção pelo *Bd* nas comunidades de anfíbios, sendo muito provável que a atividade do fungo responda diferentemente ao uso do solo e às características de micro-habitat.

A história de vida e o tipo de habitat são fatores importantes para compreender o risco e a intensidade de infecção, espécies com alta produtividade e rápido desenvolvimento podem ser mais sensíveis à infecção (JOHNSON *et al*., 2012; HAN *et al*., 2015), devido ao seu custo energético para o desenvolvimento. Os fatores ambientais são importantes métricas para avaliar a riqueza e composição de espécies de ambientes aquáticos (STENERT *et al.*, 2012). As características locais dos corpos d 'água determinam sua ocorrência, portanto, o tipo de vegetação, tamanho dos corpos d'água e a sua profundidade interferem diretamente na composição da comunidade de anfíbios (GONÇALVES, 2014). Em áreas abertas o tamanho do lago ou riacho e a profundidade da água podem contribuir para evitar a dessecação, diminuindo os riscos para girinos (SANTOS *et al*., 2007) e favorecer espécies com crescimento prolongado (CONTE e ROSSA-FERES, 2007). Além disto, as características ambientais são importantes para avaliar a ocorrência do fungo *Bd*, que apresenta variação na prevalência e carga de zoósporos em diferentes condições ambientais (SONN *et al*., 2017), estando associado à temperatura da água, temperatura do ar e pH (PIOTROWSKI *et al*., 2004). Desta forma, além das características ambientais influenciarem no estabelecimento e manutenção das espécies nos sítios reprodutivos, são também relacionadas a ocorrência da quitridiomicose. O *Bd* é prevalente tanto em anfíbios de ambientes lênticos e quanto lóticos (JOHNSON e SPEARE, 2005; LIPS *et al*., 2006; ROWLEY e ALFORD, 2007; KOLBY *et al*., 2015; RUGGERI *et al*., 2018), sendo que a prevalência e patogenicidade do *Bd* é intimamente dependente da condição climática local (KRIGER e HERO, 2007). Alterações físicas e químicas nos ambientes naturais provocadas pela interferência humana podem afetar os sítios reprodutivos dos anfíbios, e consequentemente a sua condição de resistência às doenças letais.

Técnicas para análises de *Bd* em girinos

Os sinais clínicos da quitridiomicose em anfíbios adultos são hiperplasia epidérmica e hiperqueratose (SMITH et al., 2007; FISCHER et al., 2008), com alterações na função osmorregulatória, promovendo o desequilíbrio eletrolítico e a morte (VOYLES et al., 2009).

Em girinos, são identificadas deformações na boca queratinizada (FISCHER et al., 2009), com despigmentação do bico córneo (BERGER et al., 1998).

Existem várias técnicas que são usadas para diagnosticar a infecção pelo fungo *Bd*. Uma das técnicas mais utilizadas é o PCR em tempo real. Para adultos, faz-se a coleta de material micótico com uso de *swab*, que pode ser feito ainda em campo, seguido de extração de DNA e análises de qPCR, estas em laboratório. Tais análises vão determinar a prevalência e carga de zoósporos, e geralmente segue-se o protocolo proposto por BOYLE et al. (2004) e alterações sugeridas por Lambertini et al. (2013). Esta técnica pode ser utilizada tanto para adultos quanto para girinos. Para investigar a quitridiomicose em girinos, são realizadas também análises de inspeção visual e histológica. A análise de inspeção visual pode ser realizada em campo com auxílio de uma lupa manual ou em laboratório com uso de lupa estereoscópica (LAMBERTINI et al., 2013). Ela consiste na observação do aparato bucal dos girinos, identificando indivíduos sem perda de queratina (Figura 2A) e a presença de alterações nas papilas, falhas, alterações e/ou despigmentação nas fileiras de dentes (Figura 2B e 2C) e no bico córneo (Figura 2D). Assim, de acordo com a inspeção visual é possível definir alguns padrões de deformidades. A exemplo de Navarro-Lozano et al. (2018), além de presença e ausência de deformidades, elas podem ser categorizadas em deformidades como superior/inferior e/ou parcial/total.

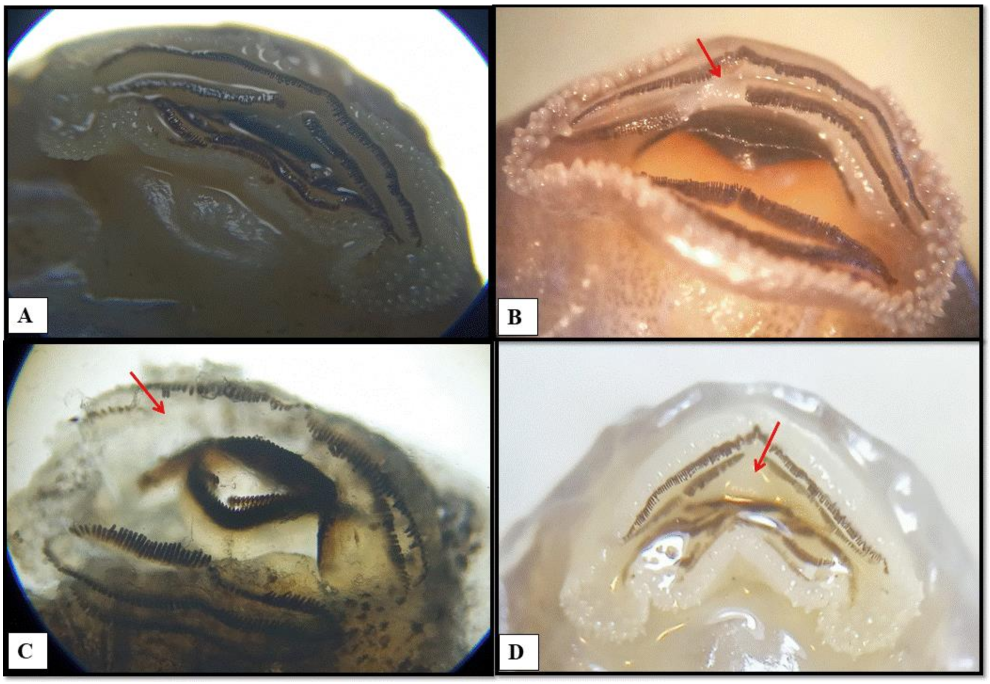

Figura 2. Aparato bucal de girino (*Boana* sp.) sem deformidade (A), aparato bucal de girino (*Leptodactylus latrans*) com alterações nas fileiras de dentes (B), aparato bucal de girino de (*Scinax* sp.), despigmentação e alterações nas fileiras de dentes e papilas (C) e aparato bucal

de girino de (*Boana* sp.), despigmentação do bico córneo (D). As setas indicam as deformidades apresentadas pelos girinos. Fonte: Fotografada e elaborada pela autora.

No entanto, determinar a presença de zoosporângios nos girinos apenas baseado nas deformidades podem resultar em falsos positivos ou falsos negativos (NAVARRO-LOZANO *et al*., 2018), sendo recomendado o uso de análises complementares. Assim, para detectar com mais confiabilidade a presença do fungo *Bd* podem ser usadas técnicas de qPCR ou análises histológicas.

As análises histológicas, são feitas diretamente no microscópico através da visualização de cortes dos tecidos do aparato bucal dos girinos (VIEIRA e TOLEDO, 2012), buscando visualizar zoosporângios presentes nos tecidos. Para isso, é necessário realizar a decapitação dos girinos, colocar em uma lâmina e fazer cortes finos com uso de um bisturi, adicionar uma gota de água destilada ou comum e cobrir com uma lamínula. Observar em microscópio com aumento de 400x (LAMBERTINI *et al*., 2013).

Em alguns táxons de anfíbios anuros os girinos apresentam disco oral, porém sem queratinização no aparato bucal (ALTIG, 2007), portanto a despigmentação nestes grupos não é considerada um atributo de infecção por *Bd*. Alguns estudos realizados mostram que a despigmentação das peças queratinizadas pode estar associada a fatores como a temperatura (RACHOWICZ, 2002) ou varia entre as diferentes fases de desenvolvimento ontogenético dos girinos (GOSNER, 1960). Porém, quando as despigmentações / deformidades foram comparadas com a presença de zoosporângios, foram encontradas forte relação entre eles, indicando que a presença de deformidades pode ser um forte indício de infecção (VIEIRA *et al*., 2013; VALÊNCIA-AGUILAR *et al*., 2016). Vieira e Toledo (2012) registraram em estudos com *Lithobates catesbeianus* que girinos menores apresentavam menores taxas de despigmentação do que girinos maiores e este mesmo padrão foi encontrado em táxons distintos. Assim, estes e outros estudos realizados com girinos mostram que eles são bons modelos para estudos de quitridiomicose, avaliando diversos fatores (bióticos e abióticos) que contribuem para a compreensão da doença entre as comunidades de anfíbios.

Ranavirose

A ranavirose está entre as doenças emergentes que acomete os anfíbios, é causada pelo patógeno ranavirus da família *Iridoviridae*, a qual apresenta cinco gêneros (JANCOVICH *et al*., 2015). O gênero Ranavirus acomete vertebrados ectotérmicos, incluindo peixes, anfíbios e répteis (JANCOVICH *et al*., 2015), impactando tanto espécies de cativeiro quanto silvestres (DUFFUS *et al*., 2015). Este grupo de patógeno tem potencial para afetar diversas espécies no mundo inteiro (GRAY e CHINCHAR, 2015) e sua ocorrência já foi registrada em todos os continentes, exceto na Antártida (DUFFUS *et al*., 2015). Devido a expansão territorial e faixa de hospedeiro, o ranavirus é considerado um patógeno emergente (DASZAK, 1999) e tem sido registrado como uma ameaça às populações de anfíbios (MILLER *et al*., 2011). Os registros indicam que o vírus já tenha afetado aproximadamente 200 espécies de ectotérmicos e o tipo de espécie do gênero conhecido como "Frog virus 3 (FV3)" (CHINCHAR *et al*., 2017).

A doença apresenta uma alta letalidade, podendo levar à extinção de uma população no local em que ela ocorre (GREEN *et al*., 2002). A susceptibilidade à doença apresenta variação nas populações e entre população com linhagem filogenética (HOVERMAN *et al*., 2011). A doença acomete principalmente os anfíbios na fase larval, de metamorfose e em adultos (CHINCHAR, 2002; GREEN *et al*., 2002).

Os sintomas mais comuns são perda de flutuabilidade; natação irregular; anorexia; inchaço (edema) do corpo, cabeça, pernas e tecidos moles internos; hemorragias externas (especialmente em torno de região ventral, periocular, região gular e pernas) e hemorragias internas ocasionais (especialmente rins, fígado e baço; MILLER *et al*., 2015). Os sintomas estão classicamente divididos em dois tipos de síndromes: a síndrome ulcerativa na pele, caracterizada por ulcerações dérmicas e a síndrome hemorrágica, caracterizada por hemorragia sistêmica no interior do corpo afetando esqueleto, músculos e órgãos viscerais (CUNNINGHAM *et al*., 1996).

No Brasil, a ocorrência da doença com eventos de mortalidade já foi registrada tanto em populações de rã-touro de cativeiro (GALLI *et al*., 2006; MAZZONI *et al*., 2009; ALENCAR, 2016; NEVES *et al*., 2016) quanto em girinos de rã-touro da família Ranidae e girinos da família Hylidae em ambientes naturais altamente alterados (RUGGERI *et al*., 2019). O estudo mais recente registrou a presença do gênero FV3 em um ranário de oito analisados na região sul e sudeste do Brasil (OLIVEIRA *et al*., 2020). Esses dados nos mostram que, assim como na América do Norte, populações nativas também podem estar sendo afetadas, principalmente pela criação e comércio da rã-touro (CUNNINGHAM *et al*., 2003).

Justificativa

Os anfíbios representam o grupo de vertebrados mais ameaçado de extinção do mundo e a Mata Atlântica, um bioma com elevada biodiversidade e endemismos, porém, altamente fragmentada por atividades antrópicas. Com isso, as modificações na paisagem podem influenciar na composição das comunidades, na seleção dos atributos de diversidade funcional dos girinos e contribuir para a incidência de patógenos que acometem os anfíbios, tornando este grupo ainda mais vulnerável.

Objetivos e hipóteses

O presente estudo teve como objetivo analisar a diversidade taxonômica das comunidades, os atributos de diversidade funcional e a incidência de patógenos (*Batrachochytrium dendrobatidis* e o ranavirus) em comunidades de girinos, verificando sua associação com o uso de solo e as características ambientais dos habitats, em remanescentes florestais do sul da Mata Atlântica.

A tese teve como hipóteses:

- Ambientes alterados formam diferentes configurações de paisagem que favorecem o aumento da riqueza de espécies, influenciando na composição de espécies.
- Descritores locais como as características físico-químicas da água e microhabitat desempenham um papel diferente como filtros ambientais em características do que na configuração da paisagem.
- As características diferem entre riachos e lagoas, uma vez que são ambientes únicos em termos de características hidrológicas e estrutura das comunidades aquáticas.
- Comunidades associadas a maiores áreas florestais apresentariam maior prevalência de *Bd*.
- Espécies que são especialistas florestais teriam maior prevalência de *Bd*.

Os objetivos da tese são:

- Analisar a associação das comunidades de girinos com as classes de paisagem;
- Avaliar a associação dos atributos de diversidade funcional dos girinos como os descritores locais de paisagem e características morfológicas do habitat e físico-químicas da água;
- Investigar a presença de ranavirus nas comunidades de girinos;
- Analisar a prevalência do fungo *Batrachochytrium dendrobatidis* (*Bd*) e sua associação com o uso do solo;
- Identificar as comunidades e espécies que estão mais susceptíveis à infecção por *Bd*.

Referências

ABREU, E. F.; CASALI, D.; COSTA-ARAÚJO, R.; GARBINO, G. S. T.; LIBARDI, G. S.; LORETTO, D.; LOSS, A. C.; MARMONTEL, M.; MORAS, L. M.; NASCIMENTO, M. C.; OLIVEIRA, M. L.; PAVAN, S. E.; TIRELLI, F. P. **Lista de Mamíferos do Brasil** (2021-2) [Data set]. Zenodo. 2021. https://doi.org/10.5281/zenodo.5802047

ALENCAR, A. L. F. **Isolamento e caracterização de estirpe de Frog virus 3-símile detectada em rãs-touro gigante (*Lithobates catesbeianus*) no Estado de São Paulo**. Dissertação de Mestrado. Universidade de São Paulo (USP), São Paulo, Brasil, p. 60, 2016.

ALTIG, R. A primer for the morphology of anuran tadpoles. **Herpetological Conservation and Biology**, v. 2, n. 1, p. 71-74, 2007.

ALTIG, R.; JOHNSTON, G. F. Guilds of anuran larvae: Relationships among modes, morphologies, and habitats. **Herpetological Monographs**, v. 3, p. 81-109, 1989.

ALTIG, R.; MCDIARMID, R. W. Body plan: Development and morphology. In: McDiarmid, R. W.; Altig, R. (eds). **Tadpoles: The Biology of Anuran Larvae**. University of Chicago Press, Chicago, p. 24-50, 1999.

AMPHIBIAWEB. University of California, Berkeley, CA, USA, 2021. Disponível em: < https://amphibiaweb.org>. Acesso em: 20 jun. 2021.

BARROWS, C. W.; ALLEN, M. F. Patterns of occurrence of reptiles across a sand dune landscape. **Journal of Arid Environments**, v. 74, n. 2, p. 186-192, 2010.

BEEBEE, T. J. C.; GRIFFITHS, R. A. The amphibian decline crisis: a watershed for conservation biology? **Biological Conservation**, v. 125, p. 271-285, 2005.

BECKER, C. G.; FONSECA, C. R.; HADDAD, C. F. B.; BATISTA, R. F.; PRADO, P. I. Habitat split and the global decline of amphibians. **Science**, v. 318, p. 1775-1777, 2007.

BECKER, C. G.; FONSECA, C. R.; HADDAD, C. F. B.; PRADO, P. I. Habitat split as a cause of local population declines of amphibians with aquatic larvae. **Conservation Biology**, v. 24, p. 287-294, 2010.

BERGER, L.; SPEARE, R.; DASZAK, P.; GREEN, D. E.; CUNNINGHAM, A. A.; GOGGIN, C. L.; SLOCOMBE, R.; RAGAN, M. A; HYATT, A. D.; MCDONALD, K. R.; HINES, H. B.; LIPS, K. R.; MARANTELLI, G.; PARKES, H. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. **Proceedings of the National Academy of Sciences of the United States of America**, v. 95, p. 9031-9036, 1998.

BERRIOZABAL-ISLAS, C.; RAMÍREZ-BAUTISTA, A.; CRUZ-ELIZALDE, R.; HERNÁNDEZ-SALINAS, U. Modification of landscape as promoter of change in structure and taxonomic diversity of reptile´s communities: an example in tropical landscape in the central region of Mexico. **Nature Conservation**, v. 28, p. 33-49, 2018.

BLAUSTEIN, A.; ROMANSIC J. M.; SCHESSELE E. A.; HAN B. A.; PESSIER A. P.; LONGCORE J. E. Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. **Conservation Biology**, v. 19, p. 1460-1468, 2005.

BLAUSTEIN, A. R.; GERVASI, S. S.; JOHNSON, P. T. J.; HOVERMAN, J. T.; BELDEN, L. K.; BRADLEY, P. W.; XIE, G. Y. Ecophysiology meets conservation: understanding the role of disease in amphibian population declines. **Philosophical Transactions of the Royal Society B**, v. 367, p. 1688-1707, 2012.

BOYLE, D. G.; BOYLE, D. B.; OLSEN, V.; MONGAN, J. A.; HYATT, A. D. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. **Diseases of Aquatic Organisms**, v. 60, p. 141-148, 2004.

BRAND, A. B.; SNODGRASS, J. W.; GALLAGHER, M. T.; CASEY, R. E.; METER, R. V. Lethal and sublethal effects of embryonic and larval exposure of Hyla versicolor to stormwater pond sediments. **Archives of Environmental Contamination and Toxicology**, v. 58, p. 325-331, 2010.

CALAÇA, A. M.; GRELLE, C. E. V. Funcional de comunidades: discussões conceituais e importantes avanços metodológicos. **Oecologia Australis**, v. 20, n. 4, p. 401-416, 2016.

CANESSA, S.; PARRIS, K. M. Multi-scale, direct and indirect effects of the urban stream syndrome on amphibian communities in streams. **PLoS ONE**, v. 8, n. 7, p. e70262, 2013. https://doi.org/10.1371/journal.pone**.** 0070262 PMID: 23922963

CARNAVAL, A. C. O. Q.; PUSCHENDORF, R.; PEIXOTO, O. L.; VERDADE, V. K.; RODRIGUES, M. T. Amphibian chytrid fungus broadly distributed in the Brazilian Atlantic Rain Forest. **EcoHealth**, v. 3, p. 41-48, 2006.

CARVALHO, T.; BECKER, C. G.; TOLEDO, L. F. Historical amphibian declines and extinctions in Brazil linked to chytridiomycosis. **Proceedings of the Royal Society of London, B: Biological Sciences**, v. 28, p. 20162254, 2017. http://dx.doi.org/10.1098/rspb.2016.2254

CATENAZZI, A.; MAY, R. V.; VREDENBURG, V. T. High prevalence of infection in tadpoles increases vulnerability to fungal pathogen in high-Andean amphibians. **Biological Conservation**, v. 159, p. 413-421, 2013.

CAYUELA, H.; LAMBREY, J.; VACHER, J. P.; MIAUD, C. Highlighting the effects of land-use change on a threatened amphibian in a human-dominated landscape. **Population Ecology**, v. 57, n. 2, p. 433-443, 2015.

CHESSON, P. Mechanisms of maintenance of species diversity. **Annual Review of Ecology and Systematics**, v. 31, p. 343-366, 2000.

CHINCHAR, V. G.; HICK, P.; INCE, I. A.; JANCOVICH, J. K.; MARSCHANG, R.; QIN, Q.; SUBRAMANIAM, K.; WALTZEK TB, WHITTINGTON R, WILLIAMS T, ZHANG Q, ICTV. Report Consortium, ICTV virus taxonomy profiles: Iridoviridae.
Journal of General Virology, v. 98, p. 890-891, 2017.

CHINCHAR, V. G. Ranaviruses (family Iridoviridae): emerging cold-blooded killers. **Archives of Virology**, v. 147, p. 447-470, 2002.

COLLINS, S. J.; FAHRIG, L. Responses of anurans to composition and configuration of agricultural landscapes. **Agriculture, Ecosystems & Environment**, v. 239, p. 399-409, 2017.

CONTE, C. E.; ROSSA-FERES, D. C. Riqueza e distribuição espaço-temporal de anuros em um remanescente de Floresta de Araucária no sudeste do Paraná. **Revista Brasileira de Zoologia**, v. 24, p. 1025-1037, 2007.

CORNWELL, W. K.; SCHWILK, D. W.; ACKERLY, D. D. A trait-based test for habitat filtering: convex hull volume. **Ecology**, v. 87, p. 1465-1471, 2006.

CUNNINGHAM, A. A.; LANGTON, T. E.; BENNET, P. M.; LEWIN, J. F.; DRURY, S. E.; GOUGH, R. E.; MACGREGOR, S. K. Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). **Philosophical Transactions of the Royal Society B**. v. 351, p. 1539-1557, 1996.

CUNNINGHAM, A. A.; DASZAK, P.; RODRIGUEZ, J. Pathogen pollution: defining a parasitological threat to biodiversity conservation. **Journal of Parasitology**, v. 89, p. S78-S83, 2003.

DALMOLIN, D. A.; TOZETTI, A. M.; PEREIRA, M. J. R. Taxonomic and functional anuran beta diversity of a subtropical metacommunity respond differentially to environmental and spatial predictors. **PLoS ONE**, v. 14, n. 11, p. e0214902, 2019. https://doi.org/10.1371/journal.pone.0214902

DALMOLIN, D. A.; TOZETTI, A. M.; PEREIRA, M. J. R. Turnover or intraspecific trait variation: explaining functional variability in a neotropical anuran metacommunity. **Aquatic Science**, v. 82, p. 62, 2020. https://doi.org/10.1007/s00027-020-00736-w

DASZAK, P.; BERGER, L.; CUNNINGHAM, A. A.; HYATT, A. D.; GREEN, D. E.; SPEARE, R. Emerging infectious diseases and amphibian population declines. **Emerging Infectious Diseases**. v. 5, p. 735-748, 1999.

DASZAK, P.; CUNNINGHAM, A. A.; HYATT, A. D. Infectious disease and amphibian population declines. **Diversity and Distributions**, v. 9, p. 141-150, 2003.

DIAS, K. S.; DOSSO, E. S.; HALL, A. S.; SCHUCH, A. P.; TOZETTI, A. M. Ecological light pollution affects anuran calling season, daily calling period, and sensitivity to light in natural Brazilian wetlands. **Science Nature**, v. 106, p. 46, 2019. https://doi.org/10.1007/s00114-019-1640-y

DÍAZ, S.; CABIDO, M. Vive la difference: plant functional diversity matters to ecosystem processes. **Trends in Ecology and Evolution**, v. 16, p. 646-655, 2001.

DÍAZ, S.; LAVOREL, S.; BELLO, F.; QUÉTIER, F.; GRIGULIS, K.; ROBSON T. M. Incorporating plant functional diversity effects in ecosystem service assessments. **Proceedings of the National Academy of Sciences USA**, v. 104, p. 20684-20689, 2007.

DINIZ, M. F.; BRITO, D. Protected areas effectiveness in maintaining viable giant anteater (*Myrmecophaga tridactyla*) populations in an agricultural frontier. **Nature Conservation**, v. 13, p. 145-151, 2015.

DINIZ, M. F.; BRITO, D. Threats to and viability of the giant anteater, *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae), in a protected Cerrado remnant encroached by urban expansion in central Brazil. **Zoological**, v. 30, n. 2, p. 151152, 2013. https://doi.org/10.1590/S198446702013000200005

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

DUELLMAN, W. E.; TRUEB, T. **Biology of Amphibia**. 2. ed. The Johns Hopkins University Press, Baltimore, 1994.

DUFFUS, A. L. J.; WALTZEK, T. B.; STÖHR, A. C.; ALLENDER, M. C.; GOTESMAN, M.; WHITTINGTON, R. J.; HICK, P.; HINES, M. K.; MARSCHANG, R. E. 2015. Distribution and host range of ranaviruses. In: GRAY, M. J.; CHINCHAR, V. G. (eds) **Ranaviruses: lethal pathogens of ectothermic vertebrates**. Springer, New York, 2015.

ERWIN, D. H. Macroevolution of ecosystem engineering, niche construction and diversity. **Trends Ecology Evolution**, v. 23, p. 304-310, 2008.

FAHRIG, L.; NUTTLE, W.K. Population ecology in spatially heterogeneous environments. In: Lovett, G. M.; Jones, C. G.; Turner, M. G.; Weathers, K. C. (eds.). **Ecosystem Function in Heterogeneous Landscapes Springer-Verlag**, New York, p. 95-118, 2005.

FIGUEIREDO, G. T.; STORTI, L. F.; LOURENÇO-DE-MORAES, R.; SHIBATTA, O. A.; ANJOS, L. Influence of microhabitat on the richness of anuran species: a case study of different landscapes in the Atlantic Forest of southern Brazil. **Anais da Academia Brasileira de Ciências**, v. 91, p. e20171023, 2019. https://doi.org/10.1590/0001-3765201920171023

FISCHER, M. C.; GARNER, T. W. J.; WALKER, S. F. Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. **Annual Review of Microbiology**, v. 63, p. 291-310, 2009.

FISCHER, M. C. Molecular toolkit unlocks life-cycle of the panzootic amphibian pathogen *Batrachochytrium dendrobatidis*. **Proceedings of the National Academy of Sciences of the United States of America**, v. 105, p. 17209-17210, 2008.

FUNDAÇÃO SOS MATA ATLÂNTICA, 2021. Disponível em <https://www.sosma.org.br/>. Acesso em: 01 jun. 2021.

GALLI, L.; PEREIRA, A.; MARQUEZ, A.; MAZZONI, R. Ranavirus detection by PCR in cultured tadpoles (*Rana catesbeiana* Shaw, 1802) from South America. **Aquaculture**, v. 257, p. 78-82, 2006.

GALINDO-LEAL, C.; CÂMARA, I. G. Atlantic Forest hotspot status: an overview**.** In: Galindo-Leal, C.; Câmara, I. G. (Eds.). The Atlantic Forest of South America: Biodiversity Status, Threats and Outlook, CABS and Island Press, Washington, pp. 3-11, 2003.

GARNER, T. W. J.; GARCIA, G.; CARROLL, B.; FISHER, M. C. Using itraconazole to clear *Batrachochytrium dendrobatidis* infection, and subsequent depigmentation of *Alytes muletensis* tadpoles. **Diseases Aquatic Organisms**, v. 83, p. 257-260, 2009.

GASTON, K. J. What is biodiversity? In: Gaston, K. J. (Ed.). **Biodiversity:** a biology of numbers and difference. Oxford: Blackwell Science, p. 1-9, 1996.

GERVASI, S. S.; URBINA, J.; HUA, J.; CHESTNUT, T.; RELYEA, R. A.; BLAUSTEIN, A. R. Experimental evidence for American bullfrog (*Lithobates catesbeianus*) susceptibility to chytrid fungus (*Batrachochytrium dendrobatidis*). **EcoHealth**, v. 10, p. 166-171, 2013.

GONÇALVES, D. D. S.; CRIVELLARI, L. B.; CONTE, C. E. Linking environmental drivers with amphibian species diversity in ponds from subtropical grasslands. **Anais da Academia Brasileira de Ciências**, v. 87, p. 3, p. 1751-1762, 2015.

GONÇALVES, D. D. S. **Diversidade e chave de identificação para girinos ocorrentes em áreas de floresta com araucária**. Dissertação de Mestrado em Ciências Biológicas. UFPR, Curitiba, 2014.

GOSNER K. L. A simplified table for staging anuran embryos and larvae with notes on identification. **Herpetologica**, v. 16, p. 183-190, 1960.

GOSWAMI, M.; BHATTACHARYYA, P.; MUKHERJEE, I.; TRIBEDI, P. Functional diversity: An important measure of ecosystem functioning. **Advances in Microbiology**, v. 7, p. 82-93, 2017.

GOUTTE, S; DUBOIS, A.; LEGENDRE, F. The importance of ambient sound level to characterize anuran habitat. **PLoS ONE**, v. 8, n. 10, p. e78020, 2013. https://doi.org/10.1371/journal.pone.0078020

GRAY, M. J.; CHINCHAR, G. V. **Ranaviruses:** Lethal Pathogens of Ectothermic Vertebrates. Springer open, p. 254, 2015. https://doi.org/10.1007/978-3-319-13755-1

GREENBERG, D. A.; PALEN, W. J.; MOOERS, A. Ø. Amphibian species traits, evolutionary history and environment predict *Batrachochytrium dendrobatidis* infection patterns, but not extinction risk. **Evolutionary Applications**, v. 10, p. 1130-1145, 2017.

GREEN, D. E.; CONVERSE, K. A.; SCHRADER, A. K. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. **Annals of the New York Academy of Sciences**, v. 969, p. 323-339, 2002.

GREENSPAN, S. E.; LAMBERTINI, C.; CARVALHO, T.; JAMES, T. Y.; TOLEDO, L. F.; HADDAD, C. F. B.; BECKER, C. G. Hybrids of amphibian chytrid show high virulence in native hosts. **Scientific Reports**, v. 8, p. 9600. 2018. DOI:10.1038/s41598-018-27828-w

GRIME, J. P. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. **Journal of ecology**, v. 86, p. 902-910, 1998.

HADDAD, C. F. B.; PRADO, C. P. A. Reproductive modes in frogs and their unexpected diversity in the Atlantic forest of Brazil. **Bioscience**, v. 55, p. 207-217, 2005.

HADDAD, C. F. B.; TOLEDO, L. F.; PRADO, C. P. A.; LOEBMANN, D.; GASPARINI, J. L.; SAZIMA, I. **Guia dos Anfíbios da Mata Atlântica** - Diversidade e Biologia. Anolis Books, São Paulo, 2013. 544 p.

HAMER, A. J.; PARRIS, K. M. Local and landscape determinants of amphibian communities in urban ponds. **Ecological Application**, v. 21, p. 378-390, 2011.

HAN, B. A.; SCHMIDT, J. P.; BOWDEN, S. E.; DRAKE, J. M. Rodent reservoirs of future zoonotic diseases. **Proceedings of the National Academy of Sciences of the United States of America**, v. 112, p. 7039-7044, 2015.

HANCE, J.; BUTLER, R. A. Brazil's Atlantic Forest (Mata Atlântica). **Mongabay**. Disponível em: https://rainforests.mongabay.com/mata-atlantica/. Acesso em 27 jan. 2022.

HAYES, T. B; FALSO, P.; GALLIPEAU, S.; STICE, M. The cause of global amphibian declines: a developmental endocrinologist's perspective. **Journal of Experimental Biology**, v. 213, n**.** 6, p. 921-933, 2010.

HILLERISLAMBERS, J.; ADLER, P. B.; HARPOLE, W. S.; LEVINE, J. M.; MAYFIELD, M. M. Rethinking community assembly through the lens of coexistence theory. **Annual review of Ecology, Evolution, and Systematics**, v. 43, p. 227-248, 2012.

HOVERMAN, J. T.; GRAY, M. J.; HAISLIP, J. T.; MILLER, D. L. Phylogeny, life history, and ecology contribute to differences in amphibian susceptibility to ranaviruses. **EcoHealth**, v. 8, n. 3, p. 301-19, 2011.

IAP – Instituto Ambiental do Paraná. **Plano de Manejo do Parque Estadual de Vila Velha**. Curitiba, Brasil, 2004.

JANCOVICH, J. K.; STECKLER, N.; WALTZEK, T. B. **Ranavirus taxonomy and phylogeny**. In: GRAY, M. J.; CHINCHAR, V. G. (eds) Ranaviruses: lethal pathogens of ectothermic vertebrates. Springer, NewYork, 2015.

JOHNSON, M. L.; SPEARE, R. Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. **Diseases of Aquatic Organisms**, v. 65, p. 181-186, 2005.

JOHNSON, P. T. J.; ROHR, J. R.; HOVERMAN, J. T.; KELLERMANNS, E.; BOWERMAN, J.; LUNDE, K. B. Living fast and dying of infection: Host life history drives interspecific variation in infection and disease risk. **Ecology letters**, v. 15, p. 235-242, 2012.

JOLY, C. A.; AIDAR, M. P. M.; KLINK, C. A.; MCGRATH, D. G.; MOREIRA, A. G.; MOUTINHO, P; NEPSTAD, D. C.; OLIVEIRA, A. A.; POTT, A.; RODAL, M. J. N.; et al. Evolution of the Brazilian phytogeography classification systems: implications for biodiversity conservation. **Ciência e Cultura**, v. 51, p. 331-348, 1999.

JOLY, C. A.; METZGER, J. P.; TABARELLI, M. Experiences from the Brazilian Atlantic Forest: ecological findings and conservation initiatives. **New Phytologist**, v. 204, p. 459-473, 2014.

KNAUTH, D. S.; MOREIRA, L. F. B.; MALTCHIK, L. Partitioning tadpole beta diversity in highland ponds with different hydroperiods. **Freshwater Science**, v. 37, n. 2, p. 380-388, 2018.

KEDDY, P. A. Assembly and response rules: two goals for predictive community ecology. **Journal of Vegetation Science**, v. 3, p.157-164, 1992.

KOLBY, J. E.; RAMIREZ, S. D.; BERGER, L.; RICHARDS-HRDLICKA, K. L.; JOCQUE, M.; SKERRATT, L. F. Terrestrial dispersion and potential environmental transmission of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). **PLoS ONE**, v. 10, p. e0125386, 2015. https://doi.org/10.1371/journal.pone.0125386

KOUMARIS, A.; FAHRIG, L. Different anuran species show different relationships to agricultural intensity. **Wetlands**, v. 36, p. 731-744, 2016.

KRAFT, N. J. B.; ADLER, P. B.; GODOY, O.; JAMES, E. C.; FULLER, S.; LEVINE, J. M. Community assembly, coexistence and the environmental filtering metaphor. **Functional Ecology**, v. 29, p. 592-599, 2015.

KRIGER, K. M.; HERO, J. M. The chytrid fungus *Batrachochytrium dendrobatidis* in non-randomly distributed across amphibian breeding habitats. **Diversity and Distributions**, v. 13, p. 781-788, 2007.

KRYNAK, K. L.; BURKE, D. J.; BENARD, M. F. Landscape and water characteristics correlate with immune defense traits across Blanchard’s cricket frog (*Acris blanchardi*) populations. **Biological Conservation**, v. 193, p. 153-167, 2016.

LAMBERTINI, C.; RODRIGUEZ, D.; BRITO, F. B.; SILVA LEITE, D. D.; TOLEDO, L. F. Diagnóstico do fungo Quitridio: *Batrachochytrium dendrobatidis*. Métodos em Herpetologia. **Herpetologia Brasileira**, v. 2, p. 12-17, 2013.

LANDE, R. Statistics and partitioning of species diversity, and similarity among multiple communities. **Oikos**, v. 76, p. 5-13, 1996.

LAUFER, G.; VAIRA, M.; PEREYRA, L. C.; AKMENTINS, M. S. The use of ephemeral reproductive sites by the explosive breeding toad *Melanophryniscus rubriventris* (Anura: Bufonidae): is it a predator cue mediated behavior? **Studies on Neotropical Fauna and Environment**, v. 50, p. 1-7, 2015.

LAVOREL, S.; GARNIER, E. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. **Functional Ecology**, v. 16, p. 545-556, 2002.

LAWTON, J. H. What do species do in ecosystems? **Oikos**, v. 71, n. 3, p. 367-374, 1994.

LIPS, K. R.; BREM, F.; BRENES, R; REEVE, J. D.; ALFORD, R. A.; VOYLES, J.; CAREY, C.; LIVO, L.; PESSIER, A. P.; COLLINS, J. P. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. **Proceedings of the National Academy of Sciences USA**, v. 103, p. 3165-3170, 2006.

LIPS, K. R.; DIFFENDORFER, J.; MENDELSON, J. R.; SEARS, M. W. Riding the wave: reconciling the roles of disease and climate change in amphibian declines. **PLoS Biology**, v. 6, p. e72, 2008. doi:10.1371/journal.pbio.0060072

LIPS, K. R. Mass mortality of the anuran fauna at an upland site in Panama. **Conservation Biology**, v. 13, n. 1, p. 117-125, 1999.

LIPS, K. R. Overview of chytrid emergence and impacts on amphibians. **Philosophical Transactions of the Royal Society B**, v. 371, p. 20150465, 2016. doi: 10.1098/rstb.2015.0465

LIU, X.; ROHR, J. R.; LI, Y. Climate, vegetation, introduced hosts and trade shape a global wildlife pandemic. **Proceedings of the Royal Society B: Biological Sciences**, v. 280, n. 1753, p. 20122506-20122506, 2013. doi:10.1098/rspb.2012.2506

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

MCDIARMID, R. W.; ALTIG, R. Introduction: the tadpole arena. In: MCDIARMID, R.W.; ALTIG, R. (eds). **Tadpoles: the biology of anuran larvae**. University of Chicago Press, Chicago, p.1-6, 1999.

MCGILL, B.; ENQUIST, B.; WEIHER, E.; WESTOBY, M. Rebuilding community ecology from functional traits. **Trends in Ecology & Evolution**, v. 21, n. 4, p. 178-185, 2006.

MCKINNEY, M. L. Effects of urbanization on species richness: a review of plants and animals. **Urban Ecosystems**. v. 11, p. 161-176, 2008.

MAGURRAN, A. **Measuring Biological Diversity**. 1st ed. Oxford, UK: Blackwell Science: pp. 256, 2004.

MARSH, D. M.; TRENHAM, P. C. Metapopulation dynamics and amphibian conservation. **Conservation Biology**, v. 15, p. 40-49, 2001.

MARTEL, A.; ADRIAENSEN, C.; SHARIFIAN-FARD, M.; SLOUIJS, A. S. V. D.; LOUETTE, G.; BAERT, K.; CROMBAGHS, B.; DEWULF, J.; PASMANS, F. The absence of zoonotic agents in invasive bullfrogs (*Lithobates catesbeianus*) in Belgium and the Netherlands. **EcoHealth**, v. 10, p. 344-347, 2013.

MAZZONI, R.; DE MESQUITA, A. J.; FLEURY, L. F. F.; DE BRITO, W. M. E. D.; NUNES, I. A.; ROBERT, J.; MORALES, H.; COELHO, A. S. G.; BARTHASSON, D. L.; GALLI, L.; et al. Mass mortality associated with a frog virus 3-like Ranavirus infection in farmed tadpoles *Rana catesbeiana* from Brazil. **Diseases of Aquatic Organisms**, v. 86, p. 181-191, 2009.

MESQUITA, A. F. C.; LAMBERTINI, C.; LYRA, M.; MALAGOLI, L. R.; JAMES, T. Y.; TOLEDO, L. F.; HADDAD, C. F. B.; BECKER, C. G. Low resistance to chytridiomycosis in direct-developing amphibians. **Scientific Reports**, v. 7, p. 16605, 2017. DOI:10.1038/s41598-017-16425-y

MICHEL, M. J. Spatial dependence of phenotype-environment associations for tadpoles in natural ponds. **Evolution and Ecology**, v. 25, p. 915-932, 2011.

MILLER, D.; GRAY, M.; STORFER, A. Ecopathology of ranaviruses infecting amphibians. **Viruses**, v.3, n. 11, p. 2351-2373, 2011.

MILLER, D. L.; PESSIER, A. P.; HICK, P.; WHITTINGTON, R. J. Comparative pathology of ranaviruses and diagnostic techniques. In: GRAY, M. J.; CHINCHAR, V. G. (eds) **Ranaviruses: lethal pathogens of ectothermic vertebrates**. Springer, New York, 2015.

MITTERMEIER, R. A.; GIL, P. R.; HOFFMANN, M.; PILGRIM, J.; BROOKS, J.; MITTERMEIER, C. G.; LAMOURUX, J.; FONSECA, G. A. B. **Hotspots revisited**: **earth's biologically richest and most endangered terrestrial ecoregions**. Washington, DC, USA: Cermex, 2005.

MITTERMEIER, R. A.; TURNER, W. R.; LARSEN, F. W.; BROOKS, T. M.; GASCON, C. Global biodiversity conservation: the critical role of hotspots. In: Zachos, F. E.; Habel, J. C. (Eds.). **Biodiversity Hotspots**. Springer, Berlin, Heidelberg, 2011, p. 3-22. http://dx.doi.org/10.1007/978-3-642-20992-5 1.

MOUCHET, M.; GUILHAUMON, F.; VILLÉGER, S.; MASON, N. W. H.; TOMASINI, J. A.; MOUILLOT, D. Towards a consensus for calculating dendrogram-based functional diversity indices. **Oikos**, v. 117, p. 794-800, 2008.

MYERS, N.; MITTERMEIER, R. A.; FONSECA, G. A. B.; KENT, J.; KENT, J. Biodiversity hotspots for conservation priorities. **Nature**, v. 403, p. 853-858, 2000.

NAVARRO-LOZANO, A.; SANCHEZ-DOMENE, D.; ROSSA-FERES, D. C.; BOSCH, J., SAWAYA, R. J. Are oral deformities in tadpoles accurate indicators of anuran chytridiomycosis? **PLoS ONE**, v. 13, n. 1, 2018. e0190955. https://doi.org/10.1371/journal.pone.0190955

NEVES, P. V.; CATROXO, M. H.; HIPOLITO, M.; RODRIGUES, C. O.; FERREIRA, C. M. Use of polyclonal antibodies, electron microscopy and histopathology to detect iridovirus like particles in bullfrogs. **Boletim do Instituto de Pesca**, v. 42, p. 523-531, 2016.

NOMURA, F.; DO PRADO, V. H. M.; DA SILVA, F. R.; BORGES, R. E.; DIAS, N. Y. N.; ROSSA-FERES, D. D. C. Are you experienced? Predator type and predator experience trade-offs in relation to tadpole mortality rates. **Journal of Zoology**, v. 284, p. 144-150, 2011.

O'HANLON, S. J.; RIEUX, A.; FARRER, R. A.; et al. Recent Asian origin of chytrid fungi causing global amphibian declines. **Science**, v. 360, p. 621-627, 2018.

OLIVEIRA FILHO, A. T.; FONTES, M. A. L. Patterns of floristic differentiation among Atlantic forests in southeastern Brazil, and the influence of climate. **Biotropica** v. 32, p. 793-810, 2000.

OLIVEIRA, C. R.; ALFAIA, S. R.; IKARI, F. L.; TAVARES, L. S.; SOUSA, R. L. M.; DE HARAKAVA, R.; FERREIRA, C. M. Detection and molecular characterization of Frog virus 3 in bullfrogs from frog farms in Brazil. **Aquaculture**, v. 516, p. 734575, 2020. Doi:10.1016/j.aquaculture.2019.734575

OLSON, D. H.; RONNENBERG, K. L. Global *Bd* Mapping Project: 2014 Update. **FrogLog**, v. 22, p. 17-21, 2014.

PELLET, J.; GUISAN, A.; PERRIN, N. A Concentric Analysis of the Impact of Urbanization on the Threatened European Tree Frog in an. **Conservation Biology**, v. 18, p. 1599-1606, 2004.

PETCHEY, O. L.; GASTON, K. J. Functional diversity: back to basics and looking forward. **Ecology Letters**, v. 9, n. 6, p. 741-758, 2006.

PILLSBURY, F. C.; MILLER, J. R. Habitat and landscape characteristics underlying anuran community structure along an urban–rural gradient. **Ecology Applied**, v. 18, n. 5, p. 1107-1118, 2008.

PIOTROWSKI, J. S.; ANNIS, S. L.; LONGCORE, J. E. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. **Mycologia**, v. 96, p. 9-15, 2004.

PIOVIA-SCOTT, J.; POPE, K. L.; LAWLER, S. P; COLE, E. M.; FOLEY, J. E. Factors related to the distribution and prevalence of the fungal pathogen *Batrachochytrium dendrobatidis* in *Rana cascadae* and other amphibians in the Klamath Mountains. **Biology Conservation**, v. 144, p. 2913-2921, 2011.

PODANI, J.; SCHMERA, D. On dendrogram-based measures of functional diversity. **Oikos**, v. 115, n. 1, p. 179-185, 2006.

POOS, M. S.; WALKER, S. C.; JACKSON D. A. Functional-diversity indices can be driven by methodological choices and species richness. **Ecology**, v. 90, n. 2, p. 341-347, 2009.

PREUSS, J. F.; GREENSPAN, S. E.; ROSSI, E. M.; GONSALES, E. M. L.; NEELY, W. J.; VALIATI, V. H.; WOODHAMS, D. C.; BECKER, C. G.; TOZETTI. A. M. Widespread pig farming practice linked to shifts in skin microbiomes and disease in pond-breeding amphibians. **Environmental Sciences and Technology**, v. 54, n. 18, p. 11301-11312, 2020.

PRIMON, E. A.; ARANTES, S. A. C. M.; SABINO, D. C. C.; AFINOVICZ, W. A. Efeito da interação Glifosato e Fósforo sobre a população de bactérias do solo na Amazônia Meridional. **27º Congresso Brasileiro de Microbiologia**, Natal-RN, v. 1101, p.1, 2013.

PURVIS, A.; HECTOR, A. Getting the measure of biodiversity. **Nature**, v. 405, p. 212-219, 2000.

RACHOWICZ, L. J. Mouthpart pigmentation in *Rana muscosa* tadpoles: seasonal changes without chytridiomycosis. **Herpetological Review**, v. 33, p. 263-265, 2002.

RACHOWICZ, L. J.; VREDENBURG, V. T. Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. **Diseases of Aquatic Organisms**, v. 61, p. 75-83, 2004.

REZENDE, C. L.; SCARANO, F. R.; ASSAD, E. D.; JOLY, C. A.; METZGER, J. P.; STRASSBURG, B. B. N.; TABARELLI, M.; FONSECA, G. A.; MITTERMEIER, R. A.

From hotspot to hopespot: An opportunity for the Brazilian Atlantic Forest. **Perspectives in Ecology and Conservation**, v. 16, n. 4, p. 208-214, 2018.

RIBEIRO, M. C.; MARTENSEN, A. C.; METZGER, J. P.; TABARELLI, M.; SCARANO, F.; FORTIN, M. J. The Brazilian Atlantic Forest: A Shrinking Biodiversity Hotspot. **Biodiversity Hotspots**, v. 8, p. 405-434, 2011.

RIBEIRO, M. C.; METZGER, J. P.; MARTENSEN, A. C.; PONZONI, F. J.; HIROTA, M. M. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. **Biology Conservation**, v. 142, p. 1141-1153, 2009.

RICKLEFS, R. E. Community Diversity: Relative Roles of Local and Regional Processes. **Science**. v. 235, n. 4785, p. 167-171, 1987.

RICKLEFS, R. E.; MILLER G. L. **Ecology**. 4th ed WH Freeman and Company, New York City, NY. 1999.

RILEY, S. P. D.; BUSTEED, G. T.; KATS, L. B.; VANDERGON, T. L.; LEE, L. F. S.; DAGIT, R. G.; KERBY, J. L.; FISHER, R. N.; SAUVAJOT, R. M. Effects of urbanization on the distribution and abundance of amphibians and invasive species in southern California streams. **Conservation Biology**, v. 19, p. 1894-1907, 2005.

ROELANTS, K.; HAAS, A.; BOSSUYT, F. Anuran radiations and the evolution of tadpole morphospace. **Proceedings of the National Academy of Sciences**, v. 108, n. 21, p. 8731-8736, 2011.

ROSSA-FERES, D.; GAREY, M. V.; CARAMASCHI, U.; NAPOLI, M. F.; NOMURA, F.; BISPO, A. A.; BRASILEIRO, C. A.; THOMÉ, M. T. C.; SAWAYA, R. J.; CONTE, C. E.; CRUZ, C. A. G.; NASCIMENTO, L. B.; GASPARINI, J. L.; ALMEIDA, A. P.; HADDAD, C. F. B. Anfíbios da Mata Atlântica: lista de espécies, histórico dos estudos, biologia e conservação. In: Monteiro-Filho, E. L. A.; Conte, C. E. (eds). **Revisões em Zoologia**: Mata Atlântica, 1 ed. Editora UFPR, Curitiba, p.237-314, 2017.

ROTHERMEL, B. B. Migratory success of juveniles: a potential constraint on connectivity for pond-breeding amphibians. **Ecology Applied**, v. 14, p. 1535-1546, 2004.

ROWLEY, J. J. L.; ALFORD, R. A. Behaviour of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. **Diseases of Aquatic Organisms**, v. 677, p. 1-9, 2007.

RUGGERI, J.; TOLEDO, L. F.; CARVALHO-E-SILVA, S. P. Stream tadpoles present high prevalence but low infection loads of *Batrachochytrium dendrobatidis* (Chytridiomycota). **Hydrobiologia**, v. 806, p. 303-311, 2018.

RUGGERI, J.; RIBEIRO, L. P.; PONTES, M. R.; TOFFOLO, C.; CANDIDO, M.; CARRIERO, M. M..; ZANELLA, N.; SOUSA, R. L. M.; TOLEDO, L. F. Discovery of Wild Amphibians Infected with Ranavirus in Brazil. **Journal of Wildlife Diseases**, v. 55, n. 4, p. 897-902. 2019.

SACCOL, S. S. A.; BOLZAN, A. M. R.; SANTOS, T. G. In the Shadow of Trees: Does Eucalyptus Afforestation Reduce Herpetofaunal Diversity in Southern Brazil? **South American Journal Herpetologic**, v. 12, n. 1, p. 42-56, 2017.

SANTOS, R. C.; DE BASTIANI, V. I. M.; MEDINA, D.; RIBEIRO, L. P.; PONTES M. R.; SILVA LEITE, D.; TOLEDO, L. F.; SOUZA FRANCO, G. M.; LUCAS, E. M. High prevalence and low intensity of infection by *Batrachochytrium dendrobatidis* in rainforest bullfrog populations in Southern Brazil. **Herpetological Conservation and Biology**, v. 15, n. 1, p. 118-130, 2020.

SANTOS, T. G.; ROSSA-FERES, D. C.; CASATTI, L. Diversidade e distribuição espaço-temporal de anuros em região com pronunciada estação seca no sudeste do Brasil. **Iheringia, Série Zoologica**, v. 97, p. 37-49, 2007.

SCHAR, C.; VIDALE, P. L.; LÜTHI, D.; FREI, C.; HÄBERLI, C.; LINIGER, M. A.; APPENZELLER, C. The role of increasing temperature variability in European summer heatwaves. **Nature**, v. 427, p. 332-336, 2004.

SCHLOEGEL, L. M.; TOLEDO, L. F.; LONGCORE, J. E.; GREENSPAN, S. E.; VIEIRA, C. A.; LEE, M.; ZHAO, S.; WANGEN, C.; FERREIRA, C. M.; HIPOLITO, M.; DAVIES, A. J.; CUOMO, C. A.; DASZAK, P.; JAMES, T. Y. Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. **Molecular Ecology**, v. 21, p. 5162-5177, 2012.

SCHMUTZER, A. C.; GRAY, M. J.; BURTON, E. C.; MILLER, D. L. Impacts of cattle on amphibian larvae and the aquatic environment. **Freshwater Biology**, v. 53, p. 2613-2625, 2008.

SEARLE, C. L.; BIGA, L. M.; SPATAFORA, J. W.; BLAUSTEIN, A. A dilution effect in the emerging amphibian pathogen *Batrachochytrium dendrobatidis*. **Proceedings of the National Academy of Sciences of the United States of America**, v. 108, n. 39, p. 16322-16326, 2011.

SIMPKINS, C. A.; KRIGER, K.; HERO, J. M. Prevalence of *Batrachochytrium dendrobatidis* on amphibians from low pH, oligotrophic waterbodies. **Herpetological Review**, v. 48, n. 4, 775-776, 2017.

SMITH, K. G.; WELDON, C.; CONRADIE, W.; PREEZ, L. H. Relationships among size, development, and *Batrachochytrium dendrobatidis* infection in African tadpoles. **Diseases of Aquatic Organisms**, v. 74, n. 2, p. 159-64, 2007.

SONN J. M.; BERMAN, S.; RICHARDS-ZAWACKI, C. L. Influence of temperature on chytridiomycosis in vivo. **EcoHealth**, v. 14, p. 762-770, 2017.

STENERT, C.; BACCA, R. C.; MORAES, A. B.; A´VILA, A. C.; MALTCHIK, L. Negative effects of exotic pine invasion on macroinvertebrate communities in southern Brazil coastal ponds. **Marine and Freshwater Research**, v. 63, p. 283-292, 2012.

STUART, S. N.; CHANSON, J. S.; COX, N. A.; YOUNG, B. E.; RODRIGUES, A. S. L.; FISCHMAN, L.; WALLER, R. W. Status and trends of amphibian declines and extinctions worldwide. **Science**, v. 306, n. 5702, p. 1783-1786, 2004.

RIBEIRO, M. C.; MARTENSEN, A. C.; METZGER, J. P.; TABARELLI, M.; SCARANO, F.; FORTIN, M. J. The Brazilian Atlantic Forest: A shrinking biodiversity hotspot. **Biodiversity Hotspots**, v. 8, p. 405-434, 2011.

RIBEIRO, M. C.; METZGER, J. P.; MARTENSEN, A. C.; PONZONI, F. J.; HIROTA, M. M. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. **Biological Conservation**, v. 142, p. 1141-1153, 2009.

RICKLEFS, R. E. Community diversity: relative roles of local and regional process. **Science**, v. 235, p. 167-171, 1987.

SACCOL, S. S. A.; BOLZAN, A. M. R.; SANTOS, T. G. In the shadow of trees: Does eucalyptus afforestation reduce herpetofaunal diversity in southern Brazil? **South American Journal of Herpetology**, v. 12, n. 1, p. 42-56. 2017.

SCHLEUTER, D.; DAUFRESNE, M.; MASSOL, F.; ARGILLIER, C. A user's guide to functional diversity índices. **Ecological Monographs**, v. 80, n. 3, p. 469-484, 2010.

SHERRATT, E.; ANSTIS, M.; KEOGH, J. S. Ecomorphological diversity of Australian tadpoles. **Nature Ecology and Evolution**, v. 8, n. 24, p. 12929-12939, 2018.

SHERRATT, E.; VIDAL-GARCÍA, M.; ANSTIS, M.; KEOGH, J. S. Adult frogs and tadpoles have different macroevolutionary patterns across the Australian continent. **Nature Ecology and Evolution**, v. 1, n. 9, p. 1385-1391, 2017.

SILVA, W. G. S.; METZGER, J. P.; SIMOES, S.; SIMONETTI, C. Relief influence on the spatial distribution of the Atlantic Forest cover at the Ibiúna Plateau, SP. **Brazilian Journal of Biology**, v. 67, n. 3, p. 403-411, 2007.

SOBRAL, F. L.; CIANCIARUSO, M. V. Phylogenetic and functional assembly structure: (re)assembling the community ecology on different spatial scales. **Bioscience Journal**, v. 28, p. 617-631, 2012.

TABARELLI, M.; AGUIAR, A. V.; RIBEIRO M. C.; METZGER J. P.; PERES C. A. Prospects for biodiversity conservation in the Atlantic Forest: lessons from aging human-modified landscapes. **Biological Conservation**, v. 143, p. 2328-2340, 2010.

THAKUR, M.; WRIGHT, A. Environmental Filtering, Niche Construction, and Trait Variability: The Missing Discussion. **Trends in Ecology & Evolution**. v. 32, p. 1-2, 2017.

TILMAN, D. Functional diversity. In: Levin, S. A. (Ed.). **Encyclopedia of Biodiversity**, vol. 3. Academic Press, New York, 2001, pp. 109-120.

TSCHARNTKE, T.; KLEIN, A. M.; KRUESS, A.; STEFFAN-DEWENTER, I.; THIES, C. Landscape perspectives on agricultural intensification and biodiversity a ecosystem service management. **Ecology Letters**, v. 8, p. 857-874, 2005.

VALENCIA-AGUILAR, A; TOLEDO, L. F.; VITAL, M. V.; MOTT, T. Seasonality, environmental factors, and host behavior linked to disease risk in stream-dwelling tadpoles. **Herpetologica**, v. 72, p. 98-106, 2016.

VIEIRA, C. A.; TOLEDO, L. F. Isolamento, cultivo e armazenamento do fungo quitrídio: *Batrachochytrium dendrobatidis*. **Herpetologia Brasileira**, v. 1, p. 18-19, 2012.

VIEIRA, C. A.; TOLEDO, L. F.; LONGCORE, J. E.; LONGCORE, J. R. Body length of *Hylodes cf. ornatus* and *Lithobates catesbeianus* tadpoles, depigmentation of mouthparts, and presence of *Batrachochytrium dendrobatidis* are related. **Brazilian Journal of Biology**, v. 73, p. 195-199, 2013.

VIOLLE, C.; NAVAS, M. L.; VILE, D.; KAZAKOU, E.; FORTUNELL, C.; HUM, I. Let the concept of trait be functional! **Oikos**, v. 116, p. 882-892, 2007.

VOYLES, J.; YOUNG, S.; BERGER, L.; CAMPBELL, C.; VOYLES, W. F.; DINUDOM, A.; COOK, D.; WEBB, R.; ALFORD, R. A.; SKERRATT, L. F.; SPEARE, R. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. **Science**, v. 326, p. 582-585, 2009.

WAKE, D. B.; VREDENBURG, V. T. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. **Proceedings of the National Academy of Sciences of the United States of America**, v. 105, n.1, p. 11466-11473, 2008.

WEBB, C. O.; ACKERLY, D. D.; MCPEEK, M. A.; DONOGHUE, M. J. Phylogenies and Community Ecology. **Annual Review of Ecology and Systematics**, v. 33, n. 1, p. 475-505, 2002.

WEIHER, E.; FREUND, D.; BUNTON, T.; STEFANSKI, A.; LEE, T.; BENTIVENGA, S. Advances, challenges and a developing synthesis of ecological community assembly theory. **Philosophical Transactions: Biological Sciences**, v. 366, p. 2403-2413, 2011.

WEIHER, E.; KEDDY, P. A. Assembly rules, null models, and trait dispersion: new questions from old patterns. **Oikos**, v. 74, p. 159-164, 1995.

WELLS, K. D. **The ecology and behavior of amphibians**. University of Chicago Press, 2007.

WHITTAKER, R. H. Evolution and measurement of species diversity. **Taxon**, v. 21, p. 213-251, 1972.

WOODHAMS, D.C.; ALFORD, R.A.; BRIGGS, C.J.; JOHNSON, M.; ROLLINS-SMITH, L.A. Life-history trade-offs influence disease in changing climates: strategies of an amphibian pathogen. **Ecology**, v. 89, p. 1627-1639, 2008.

CAPÍTULO 2 - ASSOCIATION BETWEEN LAND USE AND COMPOSITION OF AMPHIBIAN SPECIES IN TEMPERATE BRAZILIAN FOREST HABITATS

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Abstract.**—We evaluated the influence of landscape composition on the diversity of anurans in an Atlantic Forest habitat in southern Brazil. As natural habitat provides better conditions for the survival of amphibians, we expected to find more diverse communities in areas with greater forest cover. We sampled tadpoles in 25 waterbodies distributed in seven forest areas. We recorded 22 anuran species and richness varied from six to 12 species per area. Most of the recorded species were not forest specialists, except for Forest Treefrog (*****Boana curupi*****) and Schmidt's Spinythumb Frog (*****Crossodactylus schmidti*****). There was a significant overlap in the species composition among all waterbodies, and the Generalized Linear Mixed Model indicated that landscape configuration did not strongly affect species richness; however, land use drives the dissimilarity in the composition of the communities among waterbodies. Our main results show that forests and livestock farming influence changes in species assemblages among habitats. We reinforce the need for future studies incorporating temporal scale**

and a variety of spatial scales to assess landscape effects on species composition.

Key Words.—conservation; frog communities; landscape; tadpoles

INTRODUCTION

The increase of the human population generates demands to use larger areas for agriculture and livestock farming, as well as construct more roads and buildings, which leads to increased consumption of natural resources. Consequently, humans promote a variety of landscape modifications (Gururaja et al. 2008; Zhou et al. 2017). When roads, buildings or other urban facilities replace natural habitat, many ecological interactions can change, such as predation, competition, and host/parasite relationships (Nomura et al. 2011; Laufer et al. 2015; Preuss et al. 2020; Santos et al. 2020a). These changes can vary according to the intensity in which habitat is lost and air, soil, and water are polluted (Riley et al. 2005; Brand et al. 2010). One of the most studied negative effects of agriculture on wildlife is its role as a source of contamination by pesticides and herbicides (Koumaris and Fahrig 2016). When a forest is replaced by agriculture or cattle pasture, in addition to the aforementioned issues, habitat complexity and landscape heterogeneity decline (Machado et al. 2012; Saccol et al. 2017), which leads to a gradual reduction in species diversity (McKinney 2008; Barrows and Allen 2010). The intensification of human activity leads to the expansion of the urban areas, which become closer to natural habitats, causing a series of indirect (and less studied) impacts on fauna, such as light pollution (Dias et al. 2019), noise pollution (Pellet et al. 2004), and road kills (Diniz and Brito 2015). In sum, the consequences of human occupation listed above highlight the relevance of continued monitoring of biodiversity.

Landscape evaluation is a powerful tool to assess the risks of land use on biodiversity, and, consequently, supports environmental management. At the species level, landscape configuration affects fauna persistence (Fahrig and Nuttle 2005), which is a baseline for testing models of metapopulation and metacommunities. In general, species with low dispersal ability, such as amphibians, are more responsive to landscape changes (Schmutzer et al. 2008; Dixo and Metzger 2010; Diniz and Brito 2013; Cayuela et al. 2015). Most amphibians breed in aquatic sites, and changes in landscapes can restrain access to ponds or streams (Becker et al. 2007, 2010; Machado et al. 2012; Cayuela et al. 2015; Saccol et al. 2017; Dalmolin et al. 2019). This can promote morphological and physiological changes in tadpoles and adults (Costa et al. 2017). In addition, reduced reproductive potential can cause changes in community structure and composition (Berriozabal-Islas et al. 2018; Dalmolin et al. 2020), as well as population declines or local extinctions (Marsh and Trenham 2001; Rothermel 2004; Goutte et al. 2013). Therefore, amphibians are good model organisms for understanding the impact of landscape changes on physiology, morphology (Costa et al. 2017; Berriozabal-Islas et al. 2018), and species diversity (Becker et al. 2007; Pillsbury and Miller 2008; Nomura et al. 2011; Collins and Fahrig 2017).

The Brazilian Atlantic Forest, a biodiversity hotspot (Myers 2000; Mittermeier et al. 2005), is highly fragmented. More than 97% of its fragments are < 250 ha (Ribeiro et al. 2009; Zanella et al. 2012). Nonetheless, the Atlantic Forest harbors high anuran richness and endemism (Haddad et al. 2013). In the southern Atlantic Forest, most of the original forest areas have been replaced by agriculture, pasture, silviculture, and urban areas (Ribeiro et al. 2011). Studies on amphibians in this region have found positive associations between species diversity and characteristics of habitat heterogeneity (Gonçalves et al. 2015; Knauth et al. 2018; Figueiredo et al. 2019), but few analyses encompass sufficiently wide spatial scales to consider the effects of the landscape on species composition. Landscape analyses allow the detection of changes in land use and human occupation at scales compatible with the distribution of species or groups in question (Hamer and Parris 2011), and thus can help define local and regional conservation strategies.

Many studies have focused on the role of size and connectivity of forest fragments on species diversity; however, less attention is given to the role of surrounding environments generated by human occupation. Despite the negative effect of habitat modification, mosaic-like habitats often exhibit high environmental complexity, offering a variety of resources and physical conditions that may favor increased species richness (Connell, 1978; Shea et al. 2004). We evaluated the influence of the composition of the surrounding landscape on the diversity of waterbodies used by anurans as breeding sites in forest habitat of southern Brazil.

MATERIALS AND METHODS

Study area.—We conducted this study in an Atlantic Forest habitat in southern Brazil (Fig. 1; Supplemental Information Table S1). The sampled area consisted of mixed ombrophilous forest and seasonal forest with a variety of surrounding matrices including livestock pastures, agriculture, silviculture, and urban areas (Ribeiro et al. 2009; Pillar and Vélez 2010; https://www.sosmatatlantica.org.br). The climate is subtropical, with annual air temperatures varying from 16° C to 24° C and rainfall varies from 1,600–2,200 mm annually (Alvares et al.

2013). We selected forested areas for anuran sampling based on the following criteria: (1) presence of well-preserved forest (areas protected by law); (2) similar climatic conditions; (3) elevation between 300 m and 900 m above sea level; (4) similar topography; and (5) presence of lentic and lotic waterbodies used as breeding sites by anurans. We selected seven forest areas between the coordinates 22º30' to 33º45' S (latitude) and 48º02' to 57º40' W (longitude), where we sampled tadpoles in the waterbodies. We sampled at least one lentic and one lotic waterbody in each one of the seven areas to assess a wide variety of habitats and tadpole communities (Melo et al. 2017). We caught tadpoles in one to six waterbodies per area (total n = 25: 11 lentic, 14 lotic waterbodies; Supplemental Information Table S1). In lentic waterbodies, we performed time-limited sampling (1 h) that allowed us to cover a mean area of 386.45 ± 307.39 (standard deviation) m^2. In lotic waterbodies, we sampled 100-m-long sections for 1 h and the mean stream width was 1.76 m ± 0.79 (range, 0.60–3.50 m). The mean depth of the lentic waterbodies was 0.8 m ± 0.40 (range, 0.30–1.50 m) and of lotic waterbodies was 0.45 ± 0.12 (range, 0.15–0.70 m; Supplemental Information Table S1).

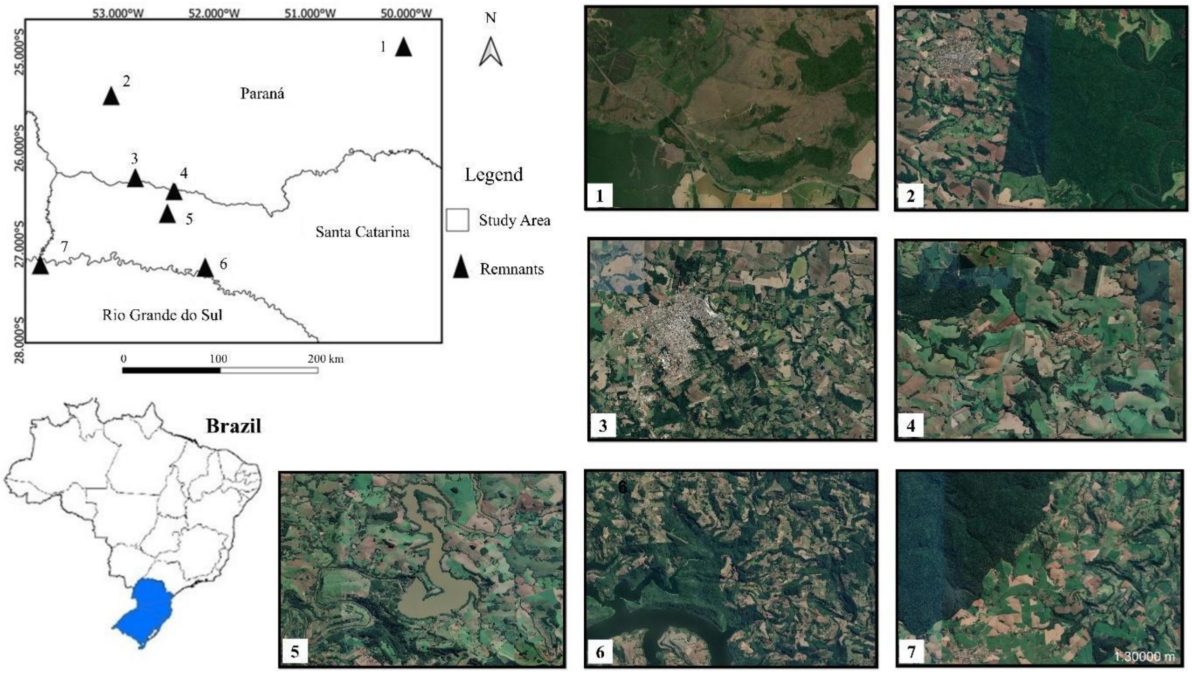

Figure 1. Study sites in southern Brazil. Satellite images obteined at https://earthexplorer.usgs.gov, 1–7 show details of the sampled areas in scale of 1:30000 m 1 = Parque Estadual de Vila Velha, Ponta Grossa/PR; 2 = Parque Estadual Rio Guarani, Três Barras/PR; 3 = Reserva Privada Enele, São Lourenço do Oeste/SC; 4 = Parque Estadual das Araucárias, São Domingos/SC; 5 = Reserva Privada Quebra-Queixo, São Domingos/SC; 6 = Parque Estadual Fritz Plaumann, Concórdia/SC; 7 = Parque Estadual do Turvo, Derrubadas/RS.

Tadpole sampling.—We sampled tadpoles from October 2018 to March 2019, which corresponds to the peak of the anuran breeding season in southern Brazil and is the most favorable period for detection of tadpoles (Both et al. 2008; Santos et al. 2008). The whole region presents a subtropical climate regime with a relatively cold and harsh winter for tropical anurans, which dramatically reduces their activity. For this reason, most species reproduce during summer when almost all species can be detected, both for adults and tadpoles (Preuss et al. 2020; Santos et al. 2020b). As the waterbodies have a wide range of geographical distribution, covering an area of about 10,500,000 ha, we were able to sample each one just once. We performed tadpole sampling from 0800–1800 using a 3-mm^2-mesh dip-net (Heyer 1976). We systematized sweepings that consisted of scouring the margins of the pond (Vasconcelos and Rossa-Feres 2005; Santos et al. 2007; Both et al. 2009; Bolzan et al. 2016). In the lotic waterbodies, we also used small dip nets to sample narrow spaces between rocks (adapted from Jordani et al. 2017). Each sample consisted of 1 h of sweeping efforts through the greatest possible variety of microhabitats.

Immediately after capture, we euthanized tadpoles by immersion in a solution of 2% lidocaine, following Brazilian Regulations (Conselho Nacional para o Controle de Experimentação Animal 2018), and subsequently transferred tadpoles to absolute ethanol. In the laboratory, we identified tadpole species with the aid of a stereomicroscope and identification keys (Machado and Maltchik 2007; Gonçalves 2014). We classified species as habitat specialists or generalists according to the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN; 2021). We considered habitat specialists as those species that are more dependent on forest habitats and have a smaller distribution area than generalists and generalists as those that are less dependent on forest habitats and have a larger geographical distribution area than specialists.

Landscape assessment.—We assessed land use based on the analysis of satellite images (Landsat 8 multispectral images, sensor Operational Land Imager from the U.S. Geological Survey [USGS] National Center, Reston, VA, USA). We classified the images from their vectorization in the software ArcGIS version 10.3 (Esri, Redlands, CA, USA), considering a 500-m-radius buffer for each sampled waterbody. We defined buffer size by the estimated size of habitats used by anurans (Semlitsch and Bodie 2003; Smith and Green 2005; Tozetti and Toledo 2005; Canessa and Parris 2013; Marques et al. 2018). We used images captured in the year of sampling (2019) and which had minimal cloud cover without significant radiometric noise. We performed the following stages of image preprocessing: (1) geometric corrections due to the inherent geometric distortions in images collected in distinct moments, by georeferencing these images; (2) atmospheric corrections aiming to reduce the interference of atmospheric scattering on the images (Soares et al. 2015); and (3) mosaicking (converting overlapping images into a single one) and contrast stretching of the different images obtained on different dates, aiming to reduce seasonal effects on the visual aspect of the image. We used the software ENVI, version 5.51 (L3Harris Geospatial, Boulder, CO, USA) for pre-processing. After the pre-processing stages, we defined the categories of land use and occupation with adjustments based on field observations. We identified the following categories (landscape variables) of land use: (1) Agriculture (cultivated areas, with soybean, corn, or wheat); (2) Aquatic environments (streams, artificial and natural ponds); (3) Forest (native forest formations in intermittent and advanced successional process); (4) Livestock pastures (extensive livestock farming); and (5) Urban area (buildings). We projected the polygons of each cover type with the reference system SIRGAS 2000, Universal Transverse Mercator (UTM) projection, zone 22S, and calculated areas in square kilometers (km²).

Data analysis.—We compared species richness based on rarefaction curves (interpolation and extrapolation method) representing standardized measures of individual abundance (Chao and Jost 2012). We calculated confidence intervals (95%) associated with the curves using the Bootstrap method (50 randomizations). For this analysis, we used the iNEXT program (iNterpolation and Extrapolation; http://chao.stat.nthu.edu.tw/wordpress/software_download/ 2016).

We tested the influence of landscape variables (Supplemental Information Table S3) on species richness using Generalized Linear Mixed-effects Models (GLMMs). We made the rarefaction and extrapolation analysis of species richness using the Inext program (iNterpelation and Extrapolation; http://chao.stat.nthu.edu.tw/wordpress/software_download/ 2016). We included waterbodies as a random variable. We evaluated the significance of each explanatory variable for species richness using the ANOVA function. We analyzed all the full models using the glm function of the lme4 package (Bates et al. 2015), in R v.3.6.0 (R Core Team 2019). We assessed the differences in species composition between the 25 waterbodies (β-diversity) and the relationship with landscape variables by using Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations (Borcard et al. 2011; Magurran and McGill 2011). PERMANOVA (which is widely used in studies focused on the beta diversity patterns) is a geometric partitioning of multivariate variation in the space of a chosen dissimilarity measure according to a given ANOVA design, this procedure uses free permutation techniques with appropriate distribution to obtain *P*-values (Anderson 2017). We measured the beta diversity by using the Bray-Curtis dissimilarity, which is appropriate for abundance data. In the PERMANOVA, we used the β diversity value as a response variable and the landscape descriptors as predictor variables. Additionally, we used a Non-Metric Multidimensional Scaling (NMDS) to visualize and interpret the differences in species composition between waterbodies. We performed the PERMANOVA and NMDS, respectively, by using the adonis and metaMDS functions of the vegan package in R v.3.6.0 (R Core Team 2019). For analysis using landscape variables on abundance data, we used 25 waterbodies to avoid overlapping buffers used to assess land use (see Supplemental Information Table S3). We used as exclusion criterion the maximum overlap of up to 40% between two buffers (see Supplemental Information Table S4).

RESULTS

We recorded 22 anuran species belonging to eight families: Bufonidae (two species; 9.1% of the total), Hylidae (12; 54.5%), Hylodidae (one; 4.5%), Leptodactylidae (three; 13.6%), Microhylidae (one; 4.5%), Odontophrynidae (one; 4.5%), Phyllomedusidae (one; 4.5%) and Ranidae (one; 4.5%), which was the non-native American Bullfrog, *Lithobates catesbeianus* (Table 1).

TABLE 1. Results of the Generalized Linear Mixed-Effect Models (GLMM), with the function glm for amphibian richness and 500-m-buffer landscape variables in 25 waterbodies (breeding sites) of Atlantic Forest habitats in southern Brazil, recorded from October 2018 to March 2019.

Variable	df	*F*	*P-value*
Forest	1, 19	0.14	0.704
Agriculture	1, 19	0.17	0.677
Livestock farming	1, 19	0.22	0.640
Urban area	1, 19	0.04	0.841
Aquatic environment	1, 19	1.15	0.296

Generalist species were numerically dominant in terms of habitat use, showing wide geographic distribution, except the Forest Treefrog (*Boana curupi*) and Schmidt´s Spinythumb Frog (*Crossodactylus schmidti*), which are forest-related species (IUCN 2021; Fig. 2). We recorded that species composition was dominated by habitat generalists, including Gladiator Treefrog (*Boana faber*), Swamp Treefrog (*Dendropsophus minutus*), American Bullfrog (*Lithobates catesbeianus*), *Physalaemus* cf. *carrizorum*, Barker Frog (*Physalaemus cuvieri*), and Snouted Treefrog (*Scinax fuscovarius*). Interpolation and extrapolation curves showed that species richness ranged from 6 to 12 among sites (Fig. 3). Landscape use did not affect species richness (Table S2), with the largest amount of richness (88%) explained by a random effect ($R^2m = 0.06$; $R^2c = 0.88$).

FIGURE 2. Two species found only in forest habitat waterbodies (breeding sites) of Atlantic Forest in southern Brazil. (A) Forest Treefrog (*Boana curupi*) and (B) Schmidt´s Spinythumb Frog (*Crossodactylus schmidti*). (Photographed by Elaine Maria Lucas).

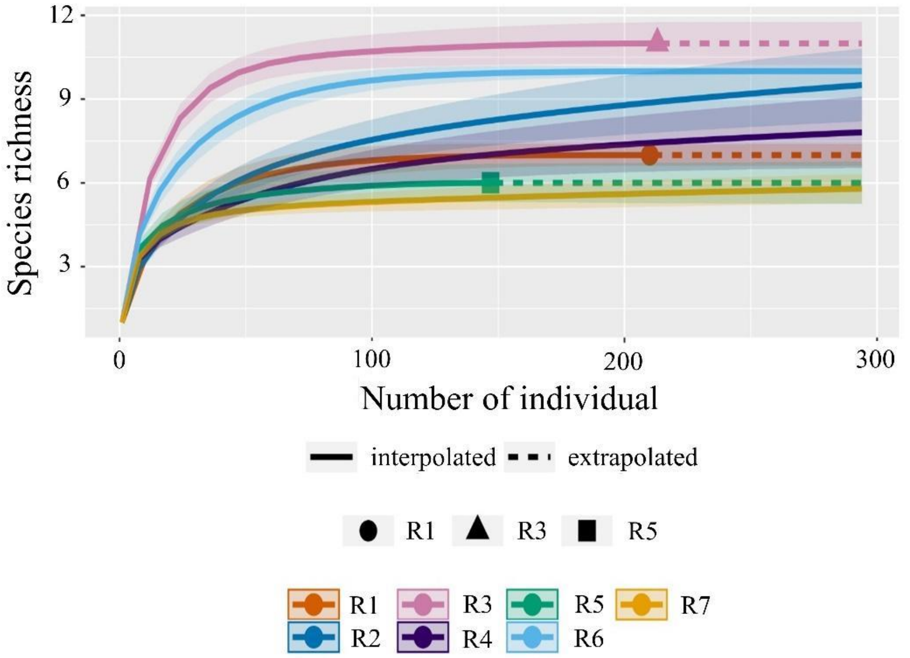

FIGURE 3. Comparison of the richness of anuran species in seven Atlantic Forest sites in southern Brazil through rarefaction (interpolation, solid lines, and extrapolation, dotted lines for ● R1, ▲R3, and ■ R5) based on the number of individuals of tadpoles in waterbodies. The acronym R1 (orange) = Parque Estadual de Vila Velha; R2 (navy blue) = Parque Estadual Rio Guarani; R3 (lilac) = Reserva Privada Enele; R4 (purple) = Parque Estadual das Araucárias; R5 (green) = Reserva Privada Quebra-Queixo; R6 (sky blue) = Parque Estadual Fritz Plaumann; R7 (yellow) = Parque Estadual do Turvo.

The dominant classes of land use were forests and livestock farming (Supplemental Information Table S2). Forests and livestock farming were the main landscape components that explained the patterns of compositional dissimilarity (beta diversity) among waterbodies (forest, $r^2 = 0.07$, $F_{1,24} = 1.77$, $P = 0.032$ livestock farming, $r^2 = 0.07$, $F_{1,24} = 2.01$, $P = 0.010$; see Table 2, Fig. 4).

TABLE 2. Results of PERMANOVA showing the contribution of landscape variables to the dissimilarity patterns in amphibian composition in the set of waterbodies (breeding sites) of Atlantic Forest habitats in southern Brazil. Asterisks (*) indicate significant P values. The abbreviation SS = sum of squares.

Variable	df	SS	*F*	*P-value*	r^2
Forest	1	0.72	1.76	0.032*	0.07
Agriculture	1	0.48	1.18	0.281	0.04
Livestock farming	1	0.81	2.01	0.010*	0.07
Urban area	1	0.49	1.21	0.182	0.04
Aquatic environment	1	0.30	0.74	0.868	0.03
Residuals	19	7.73			0.73
Total	24	10.54			1.00

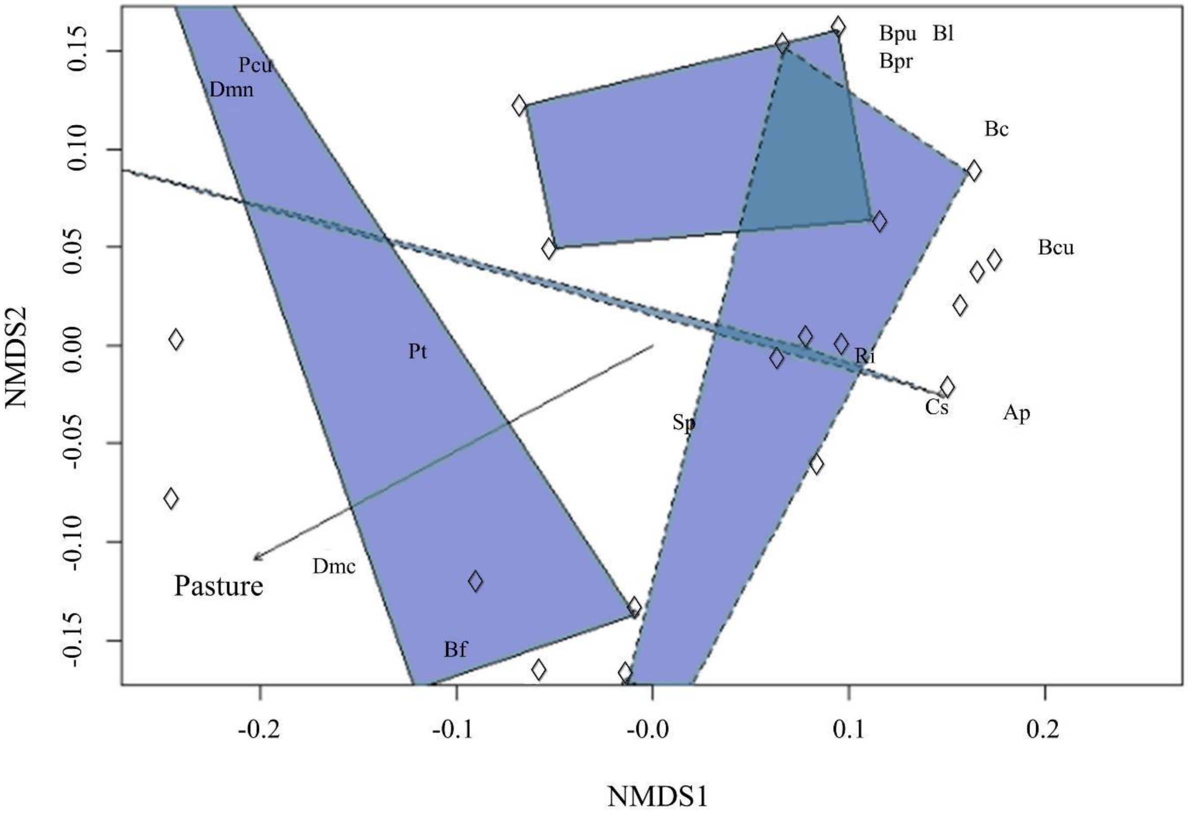

Figure 4. Non-metric Multidimensional Scaling (NMDS) based on Bray-Curtis distances showing the association between landscapes classes and composition dissimilarity (beta diversity) in 25 waterbodies. The shapes shown in blue connect the points representing different landscapes classes. The symbols (◊) represent the sampled waterbodies. The amphibian species: Ap = Green Treefrog (*Aplastodiscus perviridis*); Bc = Forest Treefrog

(*Boana curupi*); Bcu = *Boana* cf. *curupi*; Bf = Gladiator Treefrog (*Boana faber*); Bl = Striped Treefrog (*Boana leptolineata*); Bpr = Burmeister's Treefrog (*Boana prasina*); Bpu = Montevideo Treefrog (*Boana pulchella*); Cs = Schmidt's Spinythumb Frog (*Crossodactylus schmidti*); Dmc = Nova Friburgo Treefrog (*Dendropsophus microps*); Dmn = Swamp Treefrog (*Dendropsophus minutus*); Eb = Two-colored Oval Frog (*Elachistocleis bicolor*); Lc = American Bullfrog (*Lithobates catesbeianus*); Ll = Criolla Frog (*Leptodactylus latrans*); Pa = Avelino's Smooth Horned Frog (*Proceratophrys avelinoi*); Pca = *Physalaemus* cf. *carrizorum*; Pcu = Barker Frog (*Physalaemus cuvieri*); Pt = Monkey Treefrog (*Phyllomedusa tetraploidea*); Rh = Striped Toad (*Rhinella henseli*); Ri = Cururu Toad (*Rhinella icterica*); Sf = Snouted Treefrog (*Scinax fuscovarius*); Sg = Granulated Snouted Treefrog (*Scinax granulatus*); Sp = Perereca Snouted Treefrog (*Scinax perereca*).

DISCUSSION

We recorded 22 anuran species, which correspond to approximately two-thirds of the richness found in similar forest remnants in southern Brazil (Lucas and Fortes 2008; Iop et al. 2012; Bastiani and Lucas 2013). Hylidae comprised over half of species, most of which are considered generalists and found in both forest environments and grasslands or forest edges (Lucas and Fortes 2008; Almeida-Gomes and Rocha 2014; Barbosa et al. 2014; Oliveira et al. 2017). The lack of any strong association between tadpole species richness and land use suggests that frogs are not sensitive to the habitat components we measured. This result must be viewed with caution, however, because breeding site configuration, such as pond characteristics (e.g., depth, substrate, and aquatic vegetation), may play a bigger role than land use in determining tadpole species diversity (Dalmolin et al. 2020). We also must consider the differences among species regarding their breeding season. Species with a shorter reproductive season will be more difficult to detect, and thus demand high sampling effort. We assume that under-detection of such species did not influence our results, but we encourage future studies with higher number of samples per site or an all-year sampling protocol to test the assumption.

Several studies have shown that microhabitat characteristics affect amphibian richness (D’Anunciação et al. 2013; Knauth et al. 2018; Figueiredo et al. 2019; Almeida et al. 2020). Forest heterogeneity, leaf litter depth, canopy cover and presence of clearings are examples of local features that are not detectable at a landscape level but affect anuran species composition (Provete et al. 2014; Ferrante et al. 2017; Howell et al. 2019). Most local elements of the habitats support species persistence by providing fundamental resources as well as shelter (Erős et al. 2014; Landeiro et al. 2014; Datry et al. 2016; Collins and Fahrig 2017). This idea is reinforced by the fact that we found Forest Treefrog (*B. curupi*) and Schmidt's Spinythumb Frog (*C. schmidti*) only where local characteristics, such as lotic waterbodies with a rocky bottom, were present (Bastiani et al. 2012; Bastiani and Lucas 2013; Caldart et al. 2013).

The importance of fine-scale habitat features does not minimize the relevance of landscape properties, such as the amount of available habitat, for the colonization and persistence of species (Faggioni et al. 2021). Seasonal movements by frogs (e.g., to mate and lay eggs) involve many risks, and landscape changes could negatively affect them, causing a strong impact on reproductive cycles (Becker et al. 2010). Thus, we strongly recommend new landscape studies at multiple scales that consider different levels of environmental integrity.

The predominance of habitat generalists and widely distributed species (IUCN 2021) has already been documented in other forest habitats in this portion of the Atlantic Forest (Lucas and Fortes 2008; Lucas and Marocco 2011; Bastiani and Lucas 2013). Deforestation of the Atlantic Forest may favor generalist species that are adapted to open conditions, and they may replace specialized species adapted to the forest (Haddad and Prado 2005). Homogenization of biota (Ferrante et al. 2017; Nowakowski et al. 2018) could explain the lack of relationship between species composition and landscape properties that we recorded.

We highlight the fact that, in the whole sampled area, we found only two forest specialist species. We have no information about the species composition in the forest habitat we studied in past decades. Thus, we cannot determine whether we are showing a recent or a well-established scenario about the regional anuran species composition. Forest-related species tend to be more prone to population decline due to their low ability to migrate across areas where the canopy cover is absent (Howell et al. 2019). The conversion of forest to pasture, which occurred decades ago, could have contributed to the local extinction of other forest-related anuran species. These habitat changes also favor the colonization by species such as the Gladiator Treefrog (*B. faber*) and Swamp Treefrog (*D. minutus*; Aquino et al. 2004, 2010; Scott et al. 2004; Lavilla et al. 2010; Silvano et al. 2010). These species are often found in fragmented landscapes and expanding open areas (Preuss 2018; Figueiredo et al. 2019; Menin et al. 2019). We also recorded the non-native American Bullfrog (*L. catesbeianus*) in four of the seven forested areas. This species is widely distributed and well established in altered environments of the south region of Brazil (Both et al. 2011; Madalozzo et al. 2016). The process of dispersion and colonization of new localities is possibly expanding (Santos-Pereira and Rocha 2015) together with anthropogenic changes. Little is known about the persistence of populations of *L. catesbeianus* in altered environments, so this is an important subject for future studies.

The presence of livestock pasture (farming) and forests were the landscape components that best explained the dissimilarity in species composition. The role of forests and pastures in shaping amphibian species composition is relatively well understood (Haddad et al. 2013; Howell et al. 2019). At the same time, the conversion of forests into areas of pasture, agriculture, or urbanization, may limit or prevent the permanence of forest-associated species. In this sense, the composition of amphibian communities in remaining natural areas may change over time as a result of the colonization or permanence of generalist species (Ferrante et al. 2017). Future studies that examine past and present species composition and the role of changes in landscape and surrounding matrices in past decades in shaping current species assemblages would be beneficial.

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LITERATURE CITED

Almeida-Gomes, M., and C.F.D. Rocha. 2014. Landscape connectivity may explain anuran species distribution in an Atlantic Forest fragmented area. Landscape Ecology 29:29–40.

Almeida, P.C., M.T. Hartmann, and P.A. Hartmann. 2020. How riparian forest integrity influences anuran species composition: a case study in the Southern Brazil Atlantic Forest. Animal Biodiversity and Conservation 43:209–219.

Alvares, C.A., J.L. Stape, P.C. Sentelhas, J.L.M. Gonçalves, and G. Sparovek. 2013. Köppen’s climate classification map for Brazil. Meteorologische Zeitschrift 22:711–728.

Anderson, M.J. 2017. Permutational multivariate analysis of variance (PERMANOVA). Wiley Stats Ref: Statistics Reference Online. Balakrishnan, N., T. Colton, B. Everitt, W.

Piegorsch, F. Ruggeri and J.L. Teugels (Eds.). John Wiley & Sons. doi.org/10.1002/9781118445112.stat07841.

Aquino, L., A. Kwet, M.V. Segalla, V. Verdade, J. Faivovich, and D. Baldo. 2004. *Scinax perereca*. The International Union for Conservation of Nature Red List of Threatened Species 2021. https://www.iucnredlist.org.

Aquino, L., R. Bastos, S. Reichle, D. Silvano, D. Baldo, and J. Langone. 2010. *Scinax fuscovarius*. The International Union for Conservation of Nature Red List of Threatened Species 2021. https://www.iucnredlist.org.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistics Software 67:1–48.

Barbosa, A.S., M. Oliveira, A.L. Leal, C.V. Mülen, C.S. Spindler, and M. Solé. 2014. Diet of *Hypsiboas leptolineatus* (Braun and Braun, 1977) (Amphibia: Anura: Hylidae) during the breeding season. Herpetological Notes 7:505–508.

Barrows, C.W., and M.F. Allen. 2010. Patterns of occurrence of reptiles across a sand dune landscape. Journal of Arid Environments 74:186–192.

Bastiani, V.I.M., and E.M. Lucas. 2013. Anuran diversity (Amphibia, Anura) in a Seasonal Forest fragment in southern Brazil. Biota Neotropica 13:255–264.

Bastiani, V.I.M., P.C.A. Garcia, and E.M. Lucas. 2012. *Crossodactylus schmidti* Gallardo, 1961 (Anura: Hylodidae) in Santa Catarina state, southern Brazil: a new record and comments on its conservation status. Check List 8:262–63.

Becker, C.G., C.R. Fonseca, C.F.B. Haddad, and P.I. Prado. 2010. Habitat split as a cause of local population declines of amphibians with aquatic larvae. Conservation Biology 24:287–294.

Becker, C.G., C.R. Fonseca, C.F.B. Haddad, R.F. Batista, and P.I. Prado. 2007. Habitat split and the global decline of amphibians. Science 318:1775–1777.

Berriozabal-Islas, C., A. Ramírez-Bautista, R. Cruz-Elizalde, and U. Hernández-Salinas. 2018. Modification of landscape as promoter of change in structure and taxonomic diversity of reptile´s communities: an example in tropical landscape in the central region of Mexico. Nature Conservation 28:33–49.

Bolzan, A.M.R., S.A. Saccol, and T.G. Santos. 2016. Composition and diversity of anurans in the largest conservation unit in Pampa biome, Brazil. Biota Neotropica 16:e20150113. http://doi.org/10.1590/16760611-BN-2015-0113.

Borcard, D., F. Gillet, and P. Legendre. 2011. Numerical Ecology with R. Springer, New York, New York, USA.

Both, C., Í.L. Kaefer, T.G. Santos, and S.T.Z. Cechin. 2008. An austral anuran assemblage in the Neotropics: seasonal occurrence correlated with photoperiod. Journal of Natural History 42:205–222.

Both, C., R. Lingnau, A. Santos-Jr., B. Madalozzo, L.P. Lima, and T. Grant. 2011. Widespread occurrence of the American Bullfrog, *Lithobates catesbeianus* (Shaw, 1802) (Anura: Ranidae), in Brazil. South American Journal of Herpetology 6:127–134.

Both, C., M. Sole, T.G. Santos, and S.Z. Cechin. 2009. The role of spatial and temporal descriptors for neotropical tadpole communities in southern Brazil. Hydrobiologia 624:125–138.

Brand, A.B., J.W. Snodgrass, M.T. Gallagher, R.E. Casey, and R.V. Meter. 2010. Lethal and sublethal effects of embryonic and larval exposure of *Hyla versicolor* to stormwater pond sediments. Archives of Environmental Contamination and Toxicology 58:325–331.

Caldart, V.M., S. Iop, F.P. De Sá, M.C. Rocha, J.S.A. Arruda, T.G. Santos, and S.Z. Cechin. 2013. New records of *Crossodactylus schmidti* Gallardo, 1961 (Anura: Hylodidae) for the state of Rio Grande do Sul, Brazil, with data on morphometry and an updated geographic distribution map. Check List 9:1552–1555.

Canessa, S., and K.M. Parris. 2013. Multi-scale, direct and indirect effects of the urban stream syndrome on amphibian communities in streams. PLoS ONE 8:e70262. https://doi.org/10.1371/journal.pone.0070262.

Cayuela, H., J. Lambrey, J.P. Vacher, and C. Miaud. 2015. Highlighting the effects of land-use change on a threatened amphibian in a human-dominated landscape. Population Ecology 57:433–443.

Chao, A., and L. Jost. 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. Ecology 93:2533–2547.

Collins, S.J., and L. Fahrig. 2017. Responses of anurans to composition and configuration of agricultural landscapes. Agriculture, Ecosystems & Environment 239:399–409.

Conselho Nacional para o Controle de Experimentação Animal (CONCEA). 2018. Normative Resolution nº 37. National Council for Animal Experimentation Euthanasia Practice Directive. Official Diary, Brasilia, Brazil.

Connell, J.H. 1978. Diversity in tropical forests and coral reefs. Science 199:1302–1310.

Costa, R.N., M. Solé, and F. Nomura. 2017. Agropastoral activities increase fluctuating asymmetry in tadpoles of two neotropical anuran species. Austral Ecology 42:801–809.

Dalmolin, D.A., A.M. Tozetti, and M.J.R. Pereira. 2019. Taxonomic and functional anuran beta diversity of a subtropical metacommunity respond differentially to environmental and spatial predictors. PLoS ONE 14:e0214902. https://doi.org/10.1371/journal.pone.0214902.

Dalmolin, D.A., A.M. Tozetti, and M.J.R. Pereira. 2020. Turnover or intraspecific trait variation: explaining functional variability in a neotropical anuran metacommunity. Aquatic Science 82:62. https://doi.org/10.1007/s00027-020-00736-w.

D'Anunciação, P.E.R., M.F.V. Silva, L. Ferrante, D.S. Assis, T. Casagrande, A.Z.G. Coelho, B.C.S. Amâncio, T.R. Pereira, and V.X. Silva. 2013. Forest fragments surrounded by sugar cane are more inhospitable to terrestrial amphibian abundance than fragments surrounded by pasture. International Journal of Ecology 183726:8. http://dx.doi.org/10.1155/2013/183726.

Datry, T., N. Bonada, and J. Heino. 2016. Towards understanding the organization of metacommunities in highly dynamic ecological systems. Oikos 125:149–159.

Dias, K.S., E.S. Dosso, A.S. Hall, A.P. Schuch, and A.M. Tozetti. 2019. Ecological light pollution affects anuran calling season, daily calling period, and sensitivity to light in natural Brazilian wetlands. Science Nature 106:46. https://doi.org/10.1007/s00114-019-1640-y.

Diniz, M.F., and D. Brito. 2013. Threats to and viability of the Giant Anteater, *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae), in a protected Cerrado remnant encroached by urban expansion in central Brazil. Zoologia 30:151–156.

Diniz, M.F., and D. Brito. 2015. Protected areas effectiveness in maintaining viable Giant Anteater (*Myrmecophaga tridactyla*) populations in an agricultural frontier. Nature Conservation 13:145–151.

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

Erős, T., P. Sály, P. Takács, C.L. Higgins, P. Bíró, and D. Schmera. 2014. Quantifying temporal variability in the metacommunity structure of stream fishes: the influence of non-native species and environmental drivers. Hydrobiologia 722:31–43.

Faggioni, G.P., F.L. Souza, A.C. Paranhos Filho, R.M. Gamarra, and C.P.A. Prado. 2021. Amount and spatial distribution of habitats influence occupancy and dispersal of frogs at multiple scales in agricultural landscape. Austral Ecology 46:126–138.

Fahrig, L., and W.K. Nuttle. 2005. Population ecology in spatially heterogeneous environments. Pp. 95–118 *In* Ecosystem Function in Heterogeneous Landscapes. Lovett, G.M., C.G. Jones, M.G. Turner, and K.C. Weathers (Eds.). Springer-Verlag, New York, New York, USA.

Ferrante, L., F.B. Baccaro, E.B. Ferreira, M.F.O. Sampaio, T. Santos, R.C. Justino, and A. Angulo. 2017. The matrix effect: how agricultural matrices shape forest fragment structure and amphibian composition. Journal Biogeographic 44:1911–1922.

Figueiredo, G.T., L.F. Storti, R. Lourenço-De-Moraes, O.A. Shibatta, and L. Anjos. 2019. Influence of microhabitat on the richness of anuran species: a case study of different landscapes in the Atlantic Forest of southern Brazil. Anais da Academia Brasileira de Ciências 91:e20171023. https://doi.org/10.1590/0001-3765201920171023.

Gonçalves, D.D.S. 2014. Diversidade e chave de identificação para girinos ocorrentes em áreas de floresta com araucária. Ph.D. Dissertation, Universidade Federal do Paraná, Curitiba, Brasil. 131 p.

Gonçalves, D.D.S., L.B. Crivellari, and C.E. Conte. 2015. Linking environmental drivers with amphibian species diversity in ponds from subtropical grasslands. Anais da Academia Brasileira de Ciências 87:1751–1762.

Goutte, S., A. Dubois, and F. Legendre. 2013. The importance of ambient sound level to characterize anuran habitat. PLoS ONE 8:e78020. https://doi.org/10.1371/journal.pone.0078020.

Gururaja, K.V., S. Ali, and T.V. Ramachandra. 2008. Influence of land-use changes in river basins on diversity and distribution of amphibians. Pp. 33–42 *In* Environmental Education for Ecosystem Conservation. Ramachandra, T.V. (Ed.). Capital Publishing Company, New Delhi, India.

Haddad, C.F.B., and C.P.A. Prado. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. Biological Sciences 55:207–217.

Haddad, C.F.B., L.F. Toledo, C.P.A. Prado, D. Loebmann, J.L. Gasparini, and I. Sazima. 2013. Guia dos Anfíbios da Mata Atlântica: Diversidade e Biologia. Anolis Books, São Paulo, Brasil.

Hamer, A.J., and K.M. Parris. 2011. Local and landscape determinants of amphibian communities in urban ponds. Ecological Applications 21:378–390.

Heyer, W.R. 1976. Studies on larval amphibian habitat partitioning. Smithsonian Contributions to Zoology 242:1–27.

Howell, H.J., C.C. Mothes, S.L. Clements, S.V. Catania, B.B. Rothermel, and C.A. Searcy. 2019. Amphibian responses to livestock use of wetlands: new empirical data and a global review. Ecological Applications 29:e01976. https://doi.org/10.1002/eap.1976.

International Union for the Conservation of Nature (IUCN). 2021. IUCN Red List of Threatened Species, 2021. http://www.iucnredlist.org.

Iop, S., V.M. Caldart, T.G. Santos, and S.Z. Cechin. 2012. What is the role of heterogeneity and spatial autocorrelation of ponds in the organization of frog communities in southern Brazil? Zoological Studies 51:1094–1104.

Jordani, M.X., L.S. Ouchi-De-Melo, C.S. Queiroz, D.C. Rossa-Feres, and M.V. Garey. 2017. Tadpole community structure in lentic and lotic habitats: richness and diversity in the Atlantic Rainforest lowland. Herpetological Journal 27:299–306.

Knauth, D.S., L.F.B. Moreira, and L. Maltchik. 2018. Partitioning tadpole beta diversity in highland ponds with different hydroperiods. Freshwater Science 37:380–388.

Koumaris, A., and L. Fahrig. 2016. Different anuran species show different relationships to agricultural intensity. Wetlands 36:731–744.

Landeiro, V.L., F. Waldez, and M. Menin. 2014. Spatial and environmental patterns of Amazonian anurans: differences between assemblages with aquatic and terrestrial reproduction, and implications for conservation management. Nature Conservation 12:42–46.

Laufer, G., M. Vaira, L.C. Pereyra, and M.S. Akmentins. 2015. The use of ephemeral reproductive sites by the explosive breeding toad *Melanophryniscus rubriventris* (Anura: Bufonidae): is it a predator cue mediated behavior? Studies Neotropical Fauna Environment 50:1–7.

Lavilla, E., L. Aquino, A. Kwet, and D. Baldo. 2010. *Hypsiboas faber*. The International Union for Conservation of Nature Red List of Threatened Species 2021. https://www.iucnredlist.org.

Lucas, E.M., and V.B. Fortes. 2008. Frog diversity in the Floresta Nacional de Chapecó, Atlantic Forest of Southern Brazil. Biota Neotropica 8:51–61.

Lucas, E.M., and J.C. Marroco. 2011. Anurofauna (Amphibia, Anura) em um remanescente de Floresta Ombrófila Mista no Estado de Santa Catarina, Sul do Brasil. Biota Neotropica 11:377–384.

Machado, I.F., and L. Maltchik. 2007. Check-list of diversity of anurans in Rio Grande do Sul (Brazil) and a classification proposal for larval forms. Neotropical Biology and Conservation 2:101–116.

Machado, I.F., L.F.B. Moreira, and L. Maltchik. 2012. Effects of pine invasion on anurans assemblage in southern Brazil coastal ponds. Amphibia-Reptilia 33:227–237.

Madalozzo, B., C. Both, and S. Cechin. 2016. Can protected areas with agricultural edges avoid invasions? The case of bullfrogs in the Southern Atlantic Rainforest in Brazil. Zoological Studies 55:51. https://doi.org/10.6620/ZS.2016.55-51.

Magurran, A., and J. McGill. 2011. Biological Diversity. Oxford University Press, New York, New York, USA.

Marsh, D.M., and P.C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. Conservation Biology 15:40–49.

Marques, N.C.S., L. Rattis, and F. Nomura. 2018. Local environmental conditions affecting anuran tadpoles’ microhabitat choice and morphological adaptation. Marine and Freshwater Research 70:395–401.

McKinney, M.L. 2008. Effects of urbanization on species richness: a review of plants and animals. Urban Ecosystems 11:161–176.

Melo, L.S.O., T. Gonçalves-Souza, V.M. Garey, and D. Cerqueira. 2017. Tadpole species richness within lentic and lotic microhabitats: an interactive influence of environmental and spatial factors. Herpetological Journal 27:339–345.

Menin, M., R.F.B. Ferreira, I.B. Melo, M. Gordo, G.Y. Hattori, and B.S. Sant'Anna. 2019. Anuran diversity in urban and rural zones of the Itacoatiara Municipality, central Amazonia, Brazil. Acta Amazonica 49:122–130.

Mittermeier, R.A., G.A.B. Fonseca, A.B. Rylands, and K. Brandon. 2005. A brief history of biodiversity conservation in Brazil. Conservation Biology 19:601–611.

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

Nomura, F., V.H.M. Do Prado, F.R. Da Silva, R.E. Borges, N.Y.N. Dias, and D.D.C. Rossa-Feres. 2011. Are you experienced? Predator type and predator experience trade-offs in relation to tadpole mortality rates. Journal of Zoology 284:144–150.

Nowakowski, A.J., L.O. Frishkoff, M.E. Thompson, T.M. Smith, and B.D. Todd. 2018. Phylogenetic homogenization of amphibian assemblages in human-altered habitats across the globe. Proceedings of the National Academy of Sciences 115:E3454-E3462.

Oliveira, M., C.F. Moser, F.R. Avila, J.A. Bueno, and A.M Tozetti. 2017. Diet of *Aplastodiscus perviridis* Lutz 1950 (Anura, Hylidae) in subtemperate forests of southern Brazil. Neotropical Biology Conservation 12:181–184.

Pellet, J., A. Guisan, and N. Perrin. 2004. A concentric analysis of the impact of urbanization on the threatened European tree frog in an agricultural landscape. Conservation Biology 18:1599–1606.

Pillar, V.P., and E. Vélez. 2010. Extinção dos campos sulinos em Unidades de Conservação: um fenômeno natural ou um problema ético? Nature Conservation 8:84–86.

Pillsbury, F.C., and J.R. Miller. 2008. Habitat and landscape characteristics underlying anuran community structure along an urban-rural gradient. Ecological Aplications 18:1107–1118.

Preuss, J.F. 2018. Diversidade de anuros em um fragmento de Floresta Estacional no vale do rio Uruguai, sul do Brasil. Biota Amazônia 8:43–48.

Preuss, J.F., S.E, Greenspan, E.M. Rossi, E.M.L. Gonsales, W.J. Neely, V.H. Valiati, D.C. Woodhams, C.G. Becker, and A.M. Tozetti. 2020. Widespread pig farming practice linked to shifts in skin microbiomes and disease in pond-breeding amphibians. Environmental Sciences and Technology 54:11301–11312.

Provete, D.B., T. Gonçalves-Souza, M.V. Garey, I.A. Martins, and D.C. Rossa-Feres. 2014. Broad-scale spatial patterns of pond morphology and canopy cover affect the structure of Neotropical tadpole metacommunity. Hydrobiologia 734:69–79.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project. org

Ribeiro, M.C., A.C. Martensen, J.P. Metzger, M. Tabarelli, F. Scarano, and M.J. Fortin. 2011. The Brazilian Atlantic Forest: a shrinking biodiversity hotspot. Biodiversity Hotspots 8:405–434.

Ribeiro, M.C., J.P. Metzger, A.C. Martensen, F.J. Ponzoni, and M.M. Hirota. 2009. The Brazilian Atlantic Forest: how much is left, and how is the remaining forest distributed? Implications for conservation. Biology Conservation 142:1141–1153.

Riley, S.P.D., G.T. Busteed, L.B. Kats, T.L. Vandergon, L.F.S. Lee, R.G. Dagit, J.L. Kerby, R.N. Fisher, and R.M. Sauvajot. 2005. Effects of urbanization on the distribution and abundance of amphibians and invasive species in southern California streams. Conservation Biology 19:1894–1907.

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

Saccol, S.S.A., A.M.R. Bolzan, and T.G. Santos. 2017. In the shadow of trees: does eucalyptus afforestation reduce herpetofaunal diversity in Southern Brazil? South American Journal of Herpetology 12:42–56.

Santos, R.C., V.I.M. Bastiani, D. Medina, L.P. Ribeiro, M.R. Pontes, D. Silva Leite, L.F. Toledo, G.M. Souza-Franco, and E.M. Lucas. 2020a. High prevalence and low intensity of infection by *Batrachochytrium dendrobatidis* in rainforest bullfrog populations in southern Brazil. Herpetological Conservation and Biology 15:118–130.

Santos, M.B., B. Madalozzo, A.M. Tozetti, L. Loebens, and S.Z. Cechin. 2020b. Climatic dependence in the daily and seasonal calling activity of anurans from coastal wetlands of southernmost Brazil. Journal of Natural History 54:2037–2052.

Santos, T.G., L. Casatti, and D.C. Rossa-Feres. 2007. Diversidade e distribuição espaço-temporal de anuros em região com pronunciada estação seca no sudeste do Brasil. Iheringia. Série Zoologia 97:37–49.

Santos, T.G., K. Kopp, M.R. Spies, R. Trevisan, and S.Z. Cechin. 2008. Distribuição temporal e espacial de anuros em área de campos sulinos (Santa Maria, RS). Iheringia 98:244–253.

Santos-Pereira, M., and C.F.D. Rocha. 2015. Invasive Bullfrog *Lithobates catesbeianus* (Anura: Ranidae) in the Paraná state, southern Brazil: a summary of the species spread. Revista Brasileira de Zoociências 16:141–147.

Schmutzer, A.C., M.J. Gray, E.C. Burton, and D.L. Miller. 2008. Impacts of cattle on amphibian larvae and the aquatic environment. Freshwater Biology 53:2613–2625.

Scott, N., L. Aquino, D. Silvano, J. Langone, and D. Baldo. 2004. *Scinax granulatus*. The International Union for Conservation of Nature Red List of Threatened Species 2021. https://www.iucnredlist.org.

Semlitsch, R.D., and J.R. Bodie. 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. Conservation Biology 17:1219–1228.

Shea, K., S.H. Roxburgh, and E.S.J. Rauschert, 2004. Moving from pattern to process: coexistence mechanisms under intermediate disturbance regimes. Ecology Letters 7:491–508.

Silvano, D., C. Azevedo-Ramos, E. La Marca, L.A. Coloma, S. Ron, J. Langone, D. Baldo, and J. Hardy. 2010. *Dendropsophus minutus*. The International Union for Conservation of Nature Red List of Threatened Species 2021. https://www.iucnredlist.org.

Smith, M.A., and D. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography 28:110–128.

Soares, F.S., R.K. Almeida, I.B, Rubim, R.S Barros, C.B.M. Cruz, G.V. Mello, and J.A. Baptista Neto. 2015. Análise comparativa da correção atmosférica de imagem do Landsat 8: o uso do 6S e do ATCOR2. Pp 1821–1825 *In* Simpósio Brasileiro de Sensoriamento

Remoto. Gherardi, D.F.M., and L.E.O.C. Aragão (Eds.). Instituto Nacional de Pesquisas Espaciais, João Pessoa, Brasil.

Tozetti, A.M., and L.F. Toledo. 2005. Short-term movement and retreat sites of *Leptodactylus labyrinthicus* (Anura: Leptodactylidae) during the breeding season: a spool-and-line tracking study. Journal of Herpetology 39:640–644.

Vasconcelos, T.S., and D.C. Rossa-Feres. 2005. Diversidade, distribuição espacial e temporal de anfíbios anuros (Amphibia, Anura) na região noroeste do Estado de São Paulo. Biota Neotropica 5:1–14.

Zanella, L., R.A.T. Borém, C.G. Souza, H.M.R. Alves, and F.M. Borém. 2012. Atlantic Forest fragmentation analysis and landscape restoration management scenarios. Nature Conservation 10:57–63.

Zhou, W., S. Zhang, W. Yu, J. Wang, and W. Wang. 2017. Effects of urban expansion on forest loss and fragmentation in six megaregions, China. Remote Sensing 9(10), 991; https://doi.org/10.3390/rs9100991.

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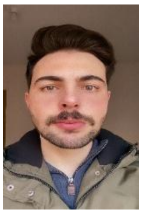

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Supplemental Information

ASSOCIATION BETWEEN LAND USE AND COMPOSITION OF AMPHIBIAN SPECIES IN TEMPERATE BRAZILIAN FOREST HABITATS

ROSELI COELHO DOS SANTOS, DIEGO ANDERSON DALMOLIN, DIEGO BRUM, MAURICIO ROBERTO VERONEZ, ELAINE MARIA LUCAS AND ALEXANDRO MARQUES TOZETTI

The following material is provided by the authors and was not subjected to peer review or editing by Herpetological Conservation and Biology.

TABLE S1. Description of waterbodies sampled in the tadpole collection in Atlantic Forest habitats in southern Brazil from October 2018 to March 2019. VV = Parque Estadual de Vila Velha; RG = Parque Estadual Rio Guarani; RE = Reserva Privada Enele; PA = Parque Estadual das Araucárias; QQ = Reserva Privada Quebra-Queixo; FP = Parque Estadual Fritz Plaumann; PT = Parque Estadual do Turvo.

Waterbody	Study area	Area	State	Geographic coordinates	Sample point	Description of the place
1	1	VV	PR	25°13'44.23"S 50° 2'8.87"O	Pond	Pond, with grassy and aquatic vegetation, in intermittent forest and close to a road, inside protected area.
2	1	VV	PR	25°14'43.39"S 50° 0'59.01"O	Stream	Stream, with grassland and shrub formation, inside the protected area.
3	1	VV	PR	25°14'52.85"S 49°59'30.90"O	Stream	Stream, with grassland and shrub formation, inside protected area.
4	1	VV	PR	25°15'33.85"S 50° 1'26.77"O	Pond	Pond, with landscape of exposed soil, grass and shrubs close to intermittent vegetation, outside the protected area.
6	2	RG	PR	25°26'25.73"S 53° 9'52.66"O	Pond	Artificial pond, with the presence of intermittent forest, at forest border, inside the protected area.
7	2	RG	PR	25°26'20.44"S 53° 9'10.23"O	Stream	Stream, with the presence of forest in an advanced process of succession, inside the protected area.
8	2	RG	PR	25°26'19.53"S 53°10'11.56"O	Pond	Artificial pond, with the presence of pasture and agriculture in the surroundings, outside the protected area.

Waterbody	Study area	Area	State	Geographic coordinates	Sample point	Description of the place
10	3	RE	SC	26°22'3.39"S 52°50'28.57"O	Pond	Artificial pond, with the presence of pasture, grass and aquatic plants, inside the private reserve.
11	3	RE	SC	26°21'57.65"S 52°50'1.40"O	Stream	Second-order stream, with the presence of forest in an advanced process of succession, inside the private reserve.
13	3	RE	SC	26°22'0.88"S 52°49'40.87"O	Stream	Stream spring, with the presence of pasture, grasses and shrubs, close to urban area, outside the private reserve.
15	4	PA	SC	26°27'28.93"S 52°33'45.54"O	Stream	Stream, with the presence of intermittent forest, inside the protected area.
16	4	PA	SC	26°28'8.27"S 52°34'17.88"O	Stream	Stream, with the presence of forest in an advanced process of succession, inside the protected area.
17	4	PA	SC	26°29'1.15"S 52°33'18.43"O	Pond	Pond, with the presence of aquatic vegetation, grasses and intermittent forest in the surroundings, pasture and crops, outside the protected area.
18	4	PA	SC	26°27'6.36"S 52°33'34.69"O	Stream	Stream, with the presence of shrubby riparian forest and crops, outside the protected area.
19	5	QQ	SC	26°39'7.80"S 52°32'30.37"O	Pond	Artificial pond, with the presence of aquatic vegetation, grasses, pastures in intermittent forest and lake, outside the private reserve.

Waterbody	Study area	Area	State	Geographic coordinates	Sample point	Description of the place
21	6	FP	SC	27°17'29.56"S 52° 6'42.63"O	Stream	Stream, with the presence of forest in an advanced process of succession, inside the protected area.
22	6	FP	SC	27°17'21.45"S 52° 6'5.13"O	Pond	Artificial pond, with the presence of aquatic vegetation, grasses and intermittent forest in the surroundings, pasture and crops, outside the protected area.
23	6	FP	SC	27°17'37.40"S 52° 6'15.14"O	Stream	Stream spring, with the presence of grasses and shrubs, outside the protected area.
24	6	FP	SC	27°17'30.69"S 52° 5'32.35"O	Stream	Stream, with the presence of grasses and intermittent riparian forest, outside protected area.
25	7	PT	RS	27°13'46.71"S 53°51'1.07"O	Pond	Pond with aquatic vegetation, surrounded intermittent forest and close to road. inside protected area.
26	7	PT	RS	27°13'28.07"S 53°51'6.13"O	Pond	Pond with aquatic vegetation, surrounded intermittent forest and close to road, inside protected area.
27	7	PT	RS	27°13'58.52"S 53°51'17.21"O	Stream	Lotic water, on the edge of intermittent forest, inside protected area.
28	7	PT	RS	27°15'4.95"S 53°56'20.40"O	Pond	Semi-temporary artificial pond, surrounded by grassy vegetation. Outside protected area, in agriculture.

Waterbody	Study area	Area	State	Geographic coordinates	Sample point	Description of the place
29	7	PT	RS	27°14'30.90"S 53°50'24.61"O	Pond	Semi-temporary artificial pond, surrounded by grassy vegetation. Outside protected area, in agriculture.
30	7	PT	RS	27°14'15.39"S 53°50'49.56"O	Stream	Lotic water, on the edge of intermittent forest, outside protected area, in agriculture.

TABLE S2. Tadpole abundance and observed and rarefied richness for the 25 waterbodies (breeding sites) of Atlantic Forest habitats in southern Brazil, recorded from October 2018 to March 2019. P = Pond; S = Stream. Species listed are Common Name: Striped Toad (*Rhinella henseli*); Cururu Toad (*Rhinella icterica*); Green Treefrog (*Aplastodiscus perviridis*); Forest Treefrog (*Boana curupi*); *Boana* cf. *curupi*; Gladiator Treefrog (*Boana faber*); Striped Treefrog (*Boana leptolineata*); Burmeister's Treefrog (*Boana prasina*); Montevideo Treefrog (*Boana pulchella*); Nova Friburgo Treefrog (*Dendropsophus microps*); Swamp Treefrog (*Dendropsophus minutus*); Snouted Treefrog (Scinax fuscovarius); Granulated Snouted Treefrog (*Scinax granulatus*); Perereca Snouted Treefrog (*Scinax perereca*); Schmidt's Spinythumb Frog (*Crossodactylus schmidti*); Criolla Frog (*Leptodactylus latrans*); Barker Frog (*Physalaemus cuvieri*); *Physalaemus* cf. *carrizorum*; Two-colored Oval Frog (*Elachistocleis bicolor*); Avelino's Smooth Horned Frog (*Proceratophrys avelinoi*); Monkey Treefrog (*Phyllomedusa tetraploidea*); American Bullfrog (*Lithobates catesbeianus*).

Family/Species	**1**	**2**	**3**	**4**	**6**	**7**	**8**	**10**	**11**	**13**	**15**	**16**	**17**
	P	**S**	**S**	**P**	**P**	**S**	**P**	**P**	**S**	**S**	**S**	**S**	**P**
BUFONIDAE													
Rhinella henseli (Lutz, 1934)	0	0	0	0	0	0	0	0	12	0	0	0	0
Rhinella icterica (Spix, 1824)	0	0	0	0	400	1	0	0	0	0	0	0	0
HYLIDAE													
Aplastodiscus perviridis Lutz 1950	0	0	0	0	0	1	0	0	0	0	0	0	0
Boana cf. *curupi*	0	0	0	0	0	0	0	0	0	0	0	0	0
Boana curupi (Garcia, Faivovich and Haddad 2007)	0	0	0	0	0	0	0	0	0	0	50	98	0
Boana faber (Wied-Neuwied, 1821)	0	0	0	0	0	0	11	53	1	0	1	0	14
Boana leptolineata (Braun and Braun, 1977)	0	0	0	0	0	0	0	0	0	0	3	6	0

Boana prasina (Burmeister, 1856)	0	67	16	0	0	0	0	0	0	0	0	0	0
Boana pulchella (Duméril and Bibron, 1841)	0	9	4	0	0	0	0	0	0	0	0	0	0
Dendropsophus microps (Peters, 1872)	0	0	0	0	0	0	0	1	4	9	0	0	0
Dendropsophus minutus (Peters, 1872)	5	0	0	0	0	0	21	0	0	14	1	0	0
Scinax fuscovarius (Lutz, 1925)	0	0	0	3	0	0	28	0	0	9	0	0	0
Scinax granulatus (Peters, 1871)	0	0	3	0	0	0	3	0	0	9	2	0	0
Scinax perereca Pombal, Haddad and Kasahara, 1995	0	0	0	8	0	0	1	0	2	0	0	0	0
HYLODIDAE													
Crossodactylus schmidti Gallardo, 1961	0	0	0	0	0	124	0	0	0	0	0	0	0
LEPTODACTYLIDAE													
Leptodactylus latrans (Steffen, 1815)	0	0	0	0	0	0	0	19	0	0	0	0	0
Physalaemus cuvieri Fitzinger, 1826	6	0	0	1	0	0	0	6	0	53	0	0	0
Physalaemus cf. *carrizorum*	0	0	0	0	0	0	0	0	0	11	0	0	0
MICROHYLIDAE													
Elachistocleis bicolor (Guérin-Méneville, 1838)	0	0	0	0	0	0	2	0	0	0	0	0	0
ODONTOPHRYNIDAE													
Proceratophrys avelinoi Mercadal de Barrio and Barrio, 1993	0	0	0	0	0	0	0	0	0	0	0	0	7
PHYLLOMEDUSIDAE													
Phyllomedusa tetraploidea Pombal and Haddad, 1992	0	0	0	88	0	0	7	0	0	0	6	0	0
RANIDAE													
Lithobates catesbeianus (Shaw 1802)	0	0	0	0	0	0	25	10	0	0	0	0	0
Total abundance	11	76	23	100	400	126	98	89	19	105	63	104	21
Total richness	2	2	3	3	1	3	8	5	4	6	6	2	2
Rarefied richness	2	1.9	2.7	1.9	1.0	1.0	5.2	3.5	2.3	5.2	2.9	1.5	2.0
Total													

Continuation

TABLE S2. Tadpole abundance and observed and rarefied richness for the 25 waterbodies (breeding sites) of Atlantic Forest habitats in southern Brazil, recorded from October 2018 to March 2019. P = Pond; S = Stream. Species listed are Common Name: Striped Toad (*Rhinella henseli*); Cururu Toad (*Rhinella icterica*); Green Treefrog (*Aplastodiscus perviridis*); Forest Treefrog (*Boana curupi*); *Boana* cf. *curupi*; Gladiator Treefrog (*Boana faber*); Striped Treefrog (*Boana leptolineata*); Burmeister's Treefrog (*Boana prasina*); Montevideo Treefrog (*Boana pulchella*); Nova Friburgo Treefrog (*Dendropsophus microps*); Swamp Treefrog (*Dendropsophus minutus*); Snouted Treefrog (Scinax fuscovarius); Granulated Snouted Treefrog (*Scinax granulatus*); Perereca Snouted Treefrog (*Scinax perereca*); Schmidt's Spinythumb Frog (*Crossodactylus schmidti*); Criolla Frog (*Leptodactylus latrans*); Barker Frog (*Physalaemus cuvieri*); *Physalaemus* cf. *carrizorum*; Two-colored Oval Frog (*Elachistocleis bicolor*); Avelino's Smooth Horned Frog (*Proceratophrys avelinoi*); Monkey Treefrog (*Phyllomedusa tetraploidea*); American Bullfrog (*Lithobates catesbeianus*).

Family/Species	**18** **S**	**19** **P**	**21** **S**	**22** **P**	**23** **S**	**24** **S**	**25** **P**	**26** **P**	**27** **S**	**28** **P**	**29** **S**	**30** **S**
BUFONIDAE												
Rhinella henseli (Lutz, 1934)	0	0	0	0	0	0	0	0	0	0	0	0
Rhinella icterica (Spix, 1824)	19	0	0	0	0	0	0	0	0	0	0	0
HYLIDAE												
Aplastodiscus perviridis Lutz 1950	0	0	0	0	0	0	0	0	0	0	0	0
Boana cf. *curupi*	0	0	55	0	30	37	0	0	0	0	0	0
Boana curupi (Garcia, Faivovich and Haddad 2007)	0	0	43	0	6	41	0	0	0	0	0	0
Boana faber (Wied-Neuwied, 1821)	91	48	0	10	0	0	7	40	6	0	0	0
Boana leptolineata (Braun and Braun, 1977)	0	0	0	0	0	0	0	0	0	0	0	0

Boana prasina (Burmeister, 1856)	0	0	0	0	0	0	0	0	0	0	0	0
Boana pulchella (Duméril and Bibron, 1841)	0	0	0	0	0	0	0	0	0	0	0	0
Dendropsophus microps (Peters, 1872)	0	7	0	40	0	0	6	14	0	0	0	0
Dendropsophus minutus (Peters, 1872)	0	53	0	0	0	0	0	2	0	0	0	0
Scinax fuscovarius (Lutz, 1925)	0	9	0	5	0	0	0	0	0	0	0	0
Scinax granulatus (Peters, 1871)	0	0	0	0	0	0	0	0	0	0	0	0
Scinax perereca Pombal, Haddad and Kasahara, 1995	0	0	0	9	0	0	0	0	0	90	67	0
HYLODIDAE												
Crossodactylus schmidti Gallardo, 1961	0	0	16	0	0	0	0	0	118	0	0	136
LEPTODACTYLIDAE												
Leptodactylus latrans (Steffen, 1815)	53	0	0	0	0	0	0	0	0	0	0	0
Physalaemus cuvieri Fitzinger, 1826	0	28	0	0	0	0	0	0	0	0	0	0
Physalaemus cf. *carrizorum*	0	0	0	8	0	0	0	0	0	0	0	0
MICROHYLIDAE												
Elachistocleis bicolor (Guérin-Méneville, 1838)	0	0	0	0	0	0	0	0	0	0	0	0
ODONTOPHRYNIDAE												
Proceratophrys avelinoi Mercadal de Barrio and Barrio, 1993	0	0	0	0	0	0	0	0	0	0	0	0
PHYLLOMEDUSIDAE												
Phyllomedusa tetraploidea Pombal and Haddad, 1992	0	0	0	19	0	0	0	56	0	0	0	0
RANIDAE												
Lithobates catesbeianus (Shaw 1802)	0	2	0	8	0	0	0	0	0	0	0	0
Total abundance	163	147	114	99	36	78	13	112	124	90	67	136
Total richness	2	6	3	7	2	2	2	4	2	1	1	1
Rarefied richness	2.0	4.2	2.9	5.9	1.8	2.0	2.0	3.0	1.4	1.0	1.0	1.0
Total												2414

TABLE S3. Average area (km²) of different categories of land use surrounding the sampled waterbodies estimated on the basis of a 500-m-radius buffer, in Atlantic Forest habitats in southern Brazil, from October 2018 to March 2019.

Waterbody	Land use area (km²)				
	Agriculture	Aquatic environment	Forest	Livestock farming	Urban area
1	0.00	0.00	0.73	0.03	0.03
2	0.00	0.00	0.01	0.00	0.03
3	0.00	0.00	0.22	0.00	0.02
4	0.00	0.00	0.35	0.00	0.00
6	0.00	0.00	0.23	0.42	0.14
7	0.00	0.00	0.79	0.00	0.00
8	0.07	0.00	0.00	0.71	0.00
10	0.00	0.00	0.22	0.54	0.02
11	0.00	0.00	0.55	0.22	0.01
13	0.00	0.00	0.37	0.31	0.11
15	0.14	0.00	0.64	0.00	0.00
16	0.00	0.00	0.68	0.10	0.00
17	0.11	0.00	0.23	0.44	0.00
18	0.37	0.00	0.42	0.00	0.00
19	0.00	0.17	0.05	0.57	0.00
21	0.00	0.00	0.79	0.00	0.00
22	0.49	0.00	0.29	0.00	0.00
23	0.32	0.00	0.47	0.00	0.00
24	0.00	0.00	0.78	0.00	0.00
25	0.03	0.01	0.37	0.38	0.00
26	0.00	0.00	0.79	0.00	0.00
27	0.03	0.00	0.35	0.41	0.00
28	0.48	0.00	0.00	0.31	0.00
29	0.36	0.00	0.00	0.42	0.00
30	0.37	0.00	0.00	0.42	0.00

Table S4. Buffer area overlap among pairs of waterbodies based on 500-m-radius circular buffers, in Atlantic Forest habitats in southern Brazil, from October 2018 to March 2019. Buffer area = 785,398 m².

Waterbodies	Overlap (m²)	Overlap (%)
06 & 08	288,847	36.8
10 & 11	105,155	13.4
11 & 13	253,512	32.3
14 & 18	156,851	20.0
15 & 18	157,054	20.0
20 & 24	11,867	1.5
25 & 26	298,698	38.0
25 & 27	270,690	34.5
25 & 30	52,173	6.6
26 & 27	30,001	3.8
27 & 30	30,594	3.9
29 & 30	76,436	9.7

CAPÍTULO 3 - Trait-environment relationship in tadpole communities of the southern Atlantic Forest

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Abstract

The diversity of functional traits can be examined by comparing morphological, physiological or behavioral attributes in communities. The set of functional traits in a community could be a result of environmental filters. However, the role of the micro and macro scale environmental components over traits is complex and needs to be better understood in tadpole communities. We investigated the association between micro (local) and macro (landscape) variables and the morphological traits of tadpoles in ponds and streams in the southern region of the Brazilian Atlantic Forest. We sampled 28 waterbodies and evaluated 2861 individuals from 22 anuran species. We performed RLQ and fourth-corner analyses to determine the patterns of trait-environment relationships and which local and landscape variables influence the morphological characteristics of tadpoles. We found that morphological traits are associated mainly with physicochemical and microhabitat attributes, being distinct between ponds and streams. In ponds, tadpole traits exhibited greater association with water depth, pH, and the presence of vegetation, whereas in the streams traits were driven by several water physicochemical components and microhabitat composition. Our results indicate that local components of habitat (water characteristics and microhabitat) have a greater influence over functional traits of tadpoles than landscape in both ponds and streams. Furthermore, we also found possible adjustments in the functional traits of tadpoles related to physicochemical characteristics of the water and microhabitat.

Keywords anurans, functional information, habitat, morphology, ponds, streams

Introduction

Functional traits are the main determinants of the biology of organisms, including biochemical, physiological, morphological, developmental or behavioral mechanisms (Violle et al. 2007). Researchers argue that studies based on functional groups provide a more direct investigation of ecological responses to environmental variation, thus favoring the comprehension of the mechanisms involved in biological diversity and ecosystem functioning more directly (Hoeinghaus et al. 2007; Goswami et al. 2017). The functional characteristics of species present in a community can be measured through their value and variation in the ecosystem (Goswami et al. 2017). Functional diversity studies are applied to analyze the trait-environment relationship and are important to analyze several taxa (e.g., amphibians, plants, fishes and birds; Carvalho and Tejerina-Garro 2015; Liu et al. 2016; Arruda Almeida et al. 2018; Lescano et al. 2018; Jordani et al. 2019).

The attributes of the environment imply morphological, physiological and phenological variations at the individual level (Petchey et al. 2009), shaping communities by selecting species that have similar traits and allowing survival under certain conditions imposed by the environment (Cornwell et al. 2006; Sobral and Cianciaruso 2012). Abiotic and biotic environmental factors act together to shape communities. Many efforts have been applied to understand how environmental filtering and other forces, such as competitive exclusion, affect the species configuration in assemblages (Menezes et al. 2020). However, less effort has been put into incorporating functional diversity in community investigations. Moreover, landscape changes are a focus of study by the scientific community due to the impacts caused on the structure and dynamics of ecosystems (Haddad et al. 2015). Environmental changes (e.g., land use, nutrient availability and cycling, atmospheric composition, climate, introduction of exotic species, and overexploitation by humans) have been promoting changes in species diversity and composition (Hooper et al. 2005). Species richness, abundance, and assemblage composition are some of the basic datasets used by ecologists to describe and compare ecological communities (Gotelli and Colwell 2001). On the other hand, since functional diversity entered the scene, researchers gave a step further in understanding ecological processes generated by the interaction between organisms and their ecosystems (Tilman et al. 1997; Diaz and Cabido 2001; Petchey et al. 2004; Hooper et al. 2005). Therefore, studies of functional traits of tadpoles may help to predict how environmental changes affect many aspects of their communities.

In this sense, environmental factors, including structural characteristics of microhabitat and physicochemical characteristics of the water, could be important to explain the functional diversity of tadpoles. These environmental characteristics can directly influence the composition of aquatic communities since they are directly related to microhabitat availability, suitability for spatial use, and dispersal. Microhabitat configuration is also a driver of predation and/or competition pressures (Wellborn et al. 1996; Werner et al. 2007; Melo et al. 2018), as well as nutrient availability (Williams et al. 2008), thus enabling the distribution of trophic guilds in the water column (Queiroz et al. 2015). They can also influence the metabolism (Afonso and Eterovick 2007), morphology and physiology of tadpoles (Sipaúba-Tavares et al. 2007; Thomaz and Cunha 2010; Mansano et al. 2012, 2014; Farquharson et al. 2016), affecting their performance. This prediction is based on the influence of the landscape on the composition of the tadpole community (Santos et al. 2021) and the local characteristics of the water such as depth, presence of vegetation (Queiroz et al. 2015), substrate (Schiesari 2006; Williams et al. 2008; Thomaz and Cunha 2010), temperature (Lima et al. 2003; Maciel and Juncá 2009), pH, nitrogen in the form of ammonia, nitrite and nitrate, phosphorus and bicarbonate (Mansano et al. 2018). Moreover, tadpoles in ponds tend to occur in microhabitats with greater similarity (ex.: depth and substrate), while in streams they tend to segregate into distinct microhabitats for each species, promoting a variation in microhabitat use (Melo et al. 2018).

Tadpoles occur in a variety of freshwater habitats, including ponds, streams, phytotelmata of bromeliads and logs, as well as permanent or temporary waterbodies (Altig and McDiarmid 1999). This adaptation to different habitats can result in great variation in the tadpoles' ecological and morphofunctional characteristics, which are influenced by different selective pressures of the environment (Duellman and Trueb 1994; Sherratt et al. 2017, 2018). The different selective pressures (e.g., abiotic and biotic factors) act as environmental filters, selecting species by their morphological, physiological, behavioral and life-history traits (Violle et al. 2012). The environmental filters can act at different scales, such as micro-spatial (local habitat characteristics) and macro-spatial (landscape characteristics), determining the patterns of community assembly (Weiher et al. 2011; Violle et al. 2012). Due to their biphasic life cycle and their water-dependent physiological characteristics, amphibians form a suitable group for studies of functional diversity.

Recent studies with tadpoles have turned their attention to functional diversity

(Jordani et al. 2019; Dalmolin et al. 2020; Lipinski et al. 2020; Santos et al. 2021) and shown that environmental and spatial descriptors are determinants for taxonomic and functional composition. The functional morphology of tadpoles can be influenced by both local heterogeneity or land use at the microhabitat level and landscape at the regional level (Queiroz et al. 2015; Marques et al. 2018). Especially in tropical regions, some studies of the functional diversity of tadpoles are based on taxonomic, phylogenetic (Violle et al. 2012; Jordani et al. 2019; Lipinski et al. 2020), and metamorphosis traits (Mogali et al. 2021). Despite the evidence that environmental factors affect the expression of morphological traits, few studies investigated the relationship between morphology and environmental components in anurans (Queiroz et al. 2015). However, identifying how morphological traits respond to local environmental and landscape characteristics can provide insight into the morphological traits of tadpoles that are crucial for community assembly, mainly in the southern portion of the Atlantic Forest, which presents a considerable anuran diversity.

This study aimed to evaluate how the environment (in both local and landscape scales) acts over the functional traits of tadpoles. We expected physicochemical characteristics of the water and microhabitat to play different roles as environmental filters. We also argue that the patterns of trait-environment relationship should vary between ponds and streams since they are very different environments (in terms of hydrological characteristics). Consequently, species composition can differ to different degrees between these environments, resulting in distinct patterns of trait-environment relationship (Schriever and Lytle 2016; Jordani et al. 2017).

Methods

Study site

We carried out the study in waterbodies in Atlantic Forest habitats in southern Brazil, between coordinates 22º30' to 33º45'S (latitude) and 48º02' to 57º40'W (longitude) (Figure 1). The landscape of the region is formed by Atlantic Forest and high-altitude grasslands (Veloso and Góes-Filho 1982; SOS Mata Atlântica and INPE 2008) with a variety of surrounding matrices including urban and rural areas with the presence of pastures and agricultural plantations (Ribeiro et al. 2009; Pillar and Vélez 2010). The climate is humid subtropical (IAP 2004), with rainfall ranging from 1600 to 2200 mm per year (Alvares et al. 2013). The wavy relief is formed by plateaus, plains and escarpments

(IAP 2004), with areas of steep slopes and embedded valleys (Santa Catarina 1986). The altitude varies from 300 to 1200 m above sea level.

The selection of the sampling sites was based on the climatic, altitudinal and rainfall patterns and with a similar history of human interference. For this, we applied the following criteria for choosing sample areas: a) presence of well-preserved remnants of the Atlantic Forest (preferentially inside protected areas); b) similar climatic conditions of temperature and rainfall; c) elevation up to 900 m above the sea level; d) similar topography; and e) presence of ponds and streams used as breeding sites by anurans. Finally, the exact sampling point (waterbody), was defined based on prior knowledge of the occurrence of amphibians and of abiotic and biotic characteristics that indicate reproductive sites of anurans (shallow water, presence of floating vegetation, and abundant vegetation at the margins) (Maltchik et al. 2011; Knauth et al. 2018).

Tadpole sampling

We sampled tadpoles in 28 waterbodies (13 ponds and 15 streams), from October 2018 to March 2019. The waterbodies consisted of permanent and semi-permanent ponds and streams that were associated with forest habitats. The mean distance between the waterbodies was 2.3 km. Distance between ponds was limited by the reduced availability of high-quality habitats encompassing ponds with a similar configuration of size, shape, and surrounding vegetation. Nevertheless, considering that our study was focused on the larval stage of anurans, whose displacement is limited to the interior of waterbodies, the spatial dependence between the samples was probably minimal even when adult anurans tend to move short distances daily (Oliveira et al. 2016). Due to logistic limitations and the required distance between sites, we adopted the strategy of sampling many and more distant sites once instead of sampling a few sites many times.

Samplings were performed from 0800AM to 0600PM, with the search standardized in one hour at each waterbody, within a single anuran breeding season. We performed a single sampling in each water body. This procedure was adopted considering a large sample area (see Figure 1) and the reproductive period of amphibians in warm seasons in southern Brazil, which is favorable to detect most species (adults and tadpoles) (Santos et al. 2020).

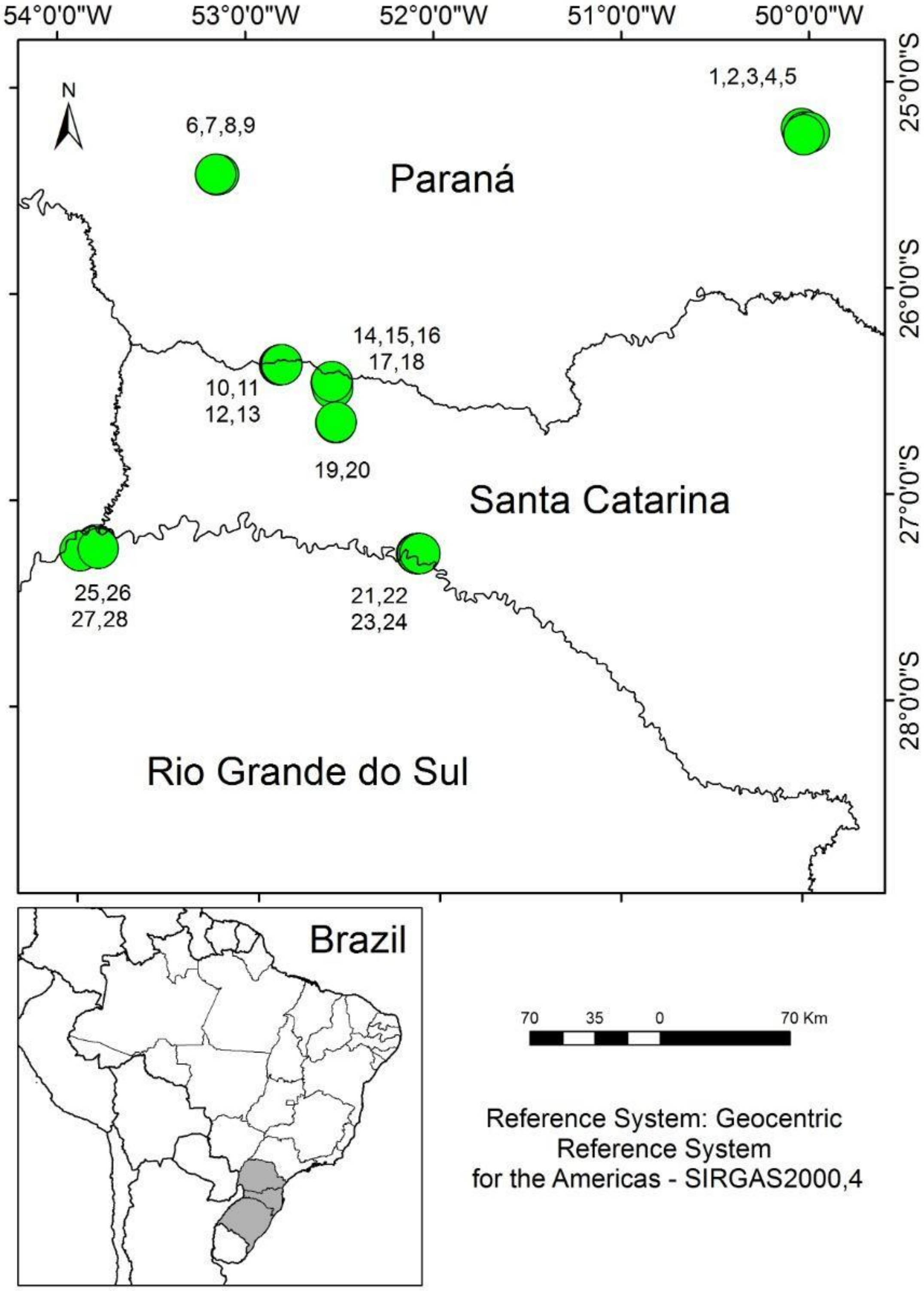


Fig. 1 Sampled waterbodies in areas of Atlantic Forest in southern Brazil. Numbers (from 1 to 28) refer to each waterbody sampled. For additional details, see Supplementary information (Figure S1).

We captured the tadpoles using a wired dip net with a mesh size of 3 mm and a diameter of 300 mm (Heyer 1976) and sweeping all available microenvironments at the margin of the waterbody, where tadpoles are more easily captured (Vasconcelos and Rossa-Feres 2005; Santos et al. 2007; Both et al. 2009; Bolzan et al. 2016). We searched through the whole margin of the ponds and, for the streams, all possible locations were searched, covering a stretch of approximately 100 m of linear margin (adapted from Jordani et al. 2017). We defined 100 m intending to achieve a sampling effort similar to that applied to ponds. This definition was based on previous tests, from which we conclude that sampling along a 100-m stretch of a stream involved an amount of time similar to sampling a single pond, and a similar number of tadpoles was captured. As the aquatic vegetation seems to affect the presence of tadpoles (Maltchik et al. 2011; Knauth et al. 2018).), we define the exact position of each transect across the stream to include the highest possible variation in marginal vegetation (density and variety of grass, shrubs, and trees). Immediately after capture, the tadpoles were euthanized, following the Brazilian regulations for the use of animals (CONCEA 2018), by immersion in a 2% lidocaine solution. Afterward, they were individualized and stored in containers with absolute ethanol. In the laboratory, we identified each specimen with the aid of a stereomicroscope and identification keys (e.g., Machado and Maltchik 2007; Gonçalves 2014).

Environment descriptors of waterbodies

We considered 33 environmental variables for each waterbody (Table 1). Variables were classified as (1) local environmental descriptors (physicochemical characteristics of the water and microhabitat configuration) and (2) landscape environmental descriptors. Regarding local descriptors, we measured 12 physicochemical characteristics of the water and 16 microhabitat components. Regarding the landscape, we evaluated five variables. All evaluated variables are listed in Table 1, followed by a brief explanation of their relevance in an ecological context.

Table 1 Environmental variables measured from sampled ponds and streams in tadpole collections. (A) Physicochemical – local scale, (B) Microhabitat – local scale and (C) Landscape-scale.

Local Scale part A – Water physicochemical analysis		
Definition of the environmental variable (Code)	**Variable**	**Ecological relevance**
Total alkalinity (total_alk)	Continuous	Indicates the concentration of carbonate and bicarbonate salts. It is associated with the formation of the plankton shell (1).
Bicarbonate (Alk_HCO3)	Continuous	Acts on pH balance (1).
Total Phosphorus (P)	Continuous	Is related to respiration and photosynthetic metabolism (phytoplankton production) (1).
Chemical Oxygen Demand (COD)	Continuous	Availability of organic matter (2).
Ammonia (NH3)	Continuous	The three are related to the excretion of aquatic organisms and the decomposition of organic matter by nitrifying bacteria (1).
Nitrate (NO3-)	Continuous	
Nitrite (NO2-)	Continuous	
pH (water_pH) = recorded in the field with multi-parameter measurer.	Continuous	Indicates the concentration of hydrogen ions (H +) and its variation is associated with the CO_2 released during phytoplankton photosynthesis (1).
Temperature (water_temp) = Water temperature in ºC, recorded in the field with multi-parameter measurer.	Continuous	Influences the metabolism of tadpoles by accelerating or inhibiting their growth (3, 4).

Dissolved oxygen (diss_oxi) = Dissolved oxygen, recorded in the field, in mg L^{-1}, with multi-parameter measurer.	Continuous	Related to tadpole breathing and decomposition of organic matter by bacteria (2).
Electrical Conductivity of water (water_conduct) = Electrical conductivity, recorded in (μScm^{-1}), with multi-parameter measurer.	Continuous	Related to the decomposition of organic matter (high) and primary production (phytoplankton; low). Food availability in water (5, 6, 7).
Transparency (water_transp) = Water transparency measured in centimeters with a Secchi disk and visual inspection to shallow.	Continuous	Related to the concentration of organic matter, sediments and phytoplankton, food resources (2).
Local Scale part B - Microhabitat		
Pond depth/stream depth (depth) = Water depth measured in centimeters, from the pond margin and the center of the stream.	Continuous	Both are related to the occurrence and persistence of species in ponds, and to competition/predation, and, consequently to reproductive success (8, 9, 10).
Pond_area/Stream_area = Area of pond/area of stream in meters, measured from the measuring tape and complemented with Google Earth.	Continuous	
Canopy opening (canopy-open) = Canopy opening recorded in percentage.	Continuous	Influences the performance of tadpoles and changes the structure of the community (11, 12).

Aquatic vegetation (aqua_veg) = Presence/absence of submerged aquatic vegetation in the pond or stream.	Categoric	Provides a greater variety of microhabitats, increasing biodiversity and the availability of resources (13).
Grasses (grass) = Presence/absence of helophyte plants in the pond or stream.	Categoric	Substrate types are related to habitat selection, species segregation, niche partition between species and availability of resources (10, 14, 15).
Rocks (rocks) = Presence/absence of rocks only at the bottom of a pond or stream.	Categoric	
Grasses and rocks (grass_rocks) = Presence/absence of rocks and herbaceous plants in the pond or stream.	Categoric	
Leaves, roots and rocks (leave_roots_rocks) = Presence/absence of leaves, roots and rocks in the pond or stream.	Categoric	
Mud (mud) = Presence/absence of mud at the bottom of a pond or stream.	Categoric	
Mud and rocks (mud_rocks) = Presence/absence of rocks and grass at the bottom of a pond or stream.	Categoric	
Roots and rocks (roots_rocks) =	Categoric	

Presence/absence of roots and rocks at the bottom of a pond or stream.		
Grasses monoculture (grass_mon) = Presence/absence of grassland type of terrestrial vegetation occupying the pond/stream margin.	Categoric	Influences the flow of organic matter, primary production (phytoplankton) and changes the structure of the community. Provides habitat, breeding sites and foraging areas (12).
Grasses and trees (grass_trees) = Presence/absence of grass and tree type of terrestrial vegetation occupying the pond/stream margin.	Categoric	
Shrubs (shrubs) = Presence/absence of shrub type of terrestrial vegetation occupying the pond/stream margin.	Categoric	
Trees (trees) = Presence/absence of tree type of terrestrial vegetation occupying the pond/stream margin.	Categoric	
Edge distance (edge distance) = Distance from the waterbody to the forest edge.	Continuous	
Landscapes-scale		
Agriculture area (Agriculture) = areas with corn, soy, wheat and pasture cultivation	Continuous	Favors the occurrence and occupation in ponds (16), influences predation, shelter and risk of desiccation (17).

Aquatic environment area (Aqua_envir) = watercourses, lakes and ponds.	Continuous
Forest area (Forest) = areas of native forest in secondary and advanced successional stage.	Continuous
Pasture area (Pasture) = areas with natural pastures, native grassy environments or livestock.	Continuous
Urbanization area (Urbanization) = secondary access roads, residential or commercial buildings.	Continuous

References: (1) Mansano et al. 2018; (2) Borges et al. 2014; (3) Lima et al. 2003; (4) Maciel and Juncá 2009; (5) Sipaúba-Tavares et al. 2007; (6) Mansano et al. 2012; (7) Mansano et al. 2014; (8) Wellborn et al. 1996; (9) Werner et al. 2007; (10) Melo et al. 2018; (11) Schiesari, 2006; (12) Williams et al. 2008; (13) Thomaz and Cunha 2010; (14) Pianka 1973; (15) Eterovick and Barata 2006; (16) Mazerolle et al. 2005; (17) Pulsford et al. 2019.

Local descriptors

Local descriptors are based on water and microhabitat evaluation. Water samples were randomly collected at each tadpole collection site, 10 cm from the waterbody's edge. We collected samples of surface water (15 cm deep) using sterilized dark plastic bottles (500 ml) that were later placed on ice before physicochemical analysis. The time between collection, transport and analysis did not exceed three days. We evaluated the total alkalinity (Total_alk), bicarbonate (Alk_HCO3), total phosphorus (P), chemical oxygen demand (COD) and nitrogen in ammonia (NH3), nitrite (NO2-) and nitrate (NO3-) (APHA 1998; Ternus et al. 2011). In the field, we collected data on pH (water_pH), temperature (water_temp) (ºC), dissolved oxygen (dissol_oxi) in mgL^{-1} and electrical conductivity (water_conduct) (µScm^{-1}) using a multiparameter meter (*Lovibond*

Sensodirect 150). Water transparency was measured with the Secchi disk by inserting it approximately 15 cm deep. For shallower water bodies where it was not possible to apply the standard Secchi disk protocol, we visually estimated the transparency, recording its maximum viewing depth. The transparency was measured at the same tadpole-collection points and was done on the banks of the ponds and at the midpoint of the streams. All data were taken by the same observer to avoid skewed weightings. These variables are considered important for tadpoles since water quality has a direct influence on their behavior and development and an indirect influence on food availability (Castaneda 2014; Zongo and Boussim 2015).

Regarding the microhabitat evaluation in each waterbody, we measured the water depth, waterbody area, and canopy opening and performed and described the vegetation and the substrate inside and outside waterbodies. The area of ponds and streams was recorded at the site using a measuring tape (in meters). For ponds, the measurement of the area was complemented with images of polygon area (*Google Earth images*), and, for streams, the area was determined by the individual width x length considering the distance of up to 100 m in each stream. Water depth was measured at three points for each waterbody at the same location where tadpoles were sampled. The canopy opening was measured using a spherical lens (*Universal clip lens*; 180º) coupled to a cell phone (*Xiaomi MI*) at the tadpole-collection point and later treated in the *GapLight* program version 2.0 (Frazer et al. 1999) and presented as a percentage. For the canopy opening, we conducted a single measurement per water body at the edge of the pond and in the center of the stream.

Substrate cover was evaluated by a systematic visual inspection. For the qualitative recording of the substrate and terrestrial vegetation, we used four quadrats of 4 m² in the interior and banks of the water bodies, with the margins of the ponds and the 100 m transects in the streams as the recording point. Finally, the distance from the forest edge was measured in meters using tape from the edge of the pond/stream to the transition point of forest vegetation with roads and/or open areas. We identified eight predominant categories of substrate cover: aquatic vegetation (aquatic_veg), grasses (grass), rocks (rocks), grasses and rocks (grass_rocks), leaves, roots and rocks (leave_roots_rocks), mud (mud), mud and rocks (mud_rocks) and roots and rocks (roots_rocks). As aquatic vegetation (aqua_veg), we consider the presence of hydrophytes and macrophytes that are completely submerged (e.g., *Elodea* sp.) and, as grasses, we consider helophytic plants that are partially submerged (e.g., Cyperaceae). Following, we recorded and classified the waterbody's terrestrial or marginal vegetation into four types: grasses (grass), grasses and trees (grass_trees), shrubs (shrubs), and trees (trees). We considered phorophytes > 2 m as trees and < 2 m as shrubs, while grasses included native herbaceous plants (e.g., Cyperaceae, Poaceae and Typhaceae) and pasture monocultures (e.g., *Brachiaria* sp.).

Landscape descriptors

We assessed land use based on the analysis of satellite images (Landsat 8 multispectral images, sensor Operational Land Imager from the U.S. Geological Survey; https://earthexplorer.usgs.gov). We used images captured in the year of sampling (2019) and that had minimal cloud cover without significant radiometric noise. We performed the following pre-processing steps of the images: 1) geometric corrections, due to the inherent geometric distortions that images collected at different times have, through the georeferencing of these images; 2) atmospheric corrections to reduce the interference of atmospheric scattering on the images (Soares et al. 2015); and 3) mosaic and enhancement of the different images in each season to reduce the seasonal effects on the visual aspect of the images. Pre-processing was done using the ENVI software, version 5.51 (L3Harris Geospatial, Boulder, Colorado, USA). After the pre-processing steps, we defined classes of land use and occupation based on on-site observations, considering the predominant use of the areas. The categories were established as Agriculture area, Aquatic environments area, Forest area, Pastures area, and Urbanization area (Table 1). We classified the images based on their vectorization in ArcGIS software version 10.3 (Esri, Redlands, California, USA), considering the buffer with a 250-m radius for each waterbody. The buffer size was based on previous studies that describe the average habitat size for amphibians, ranging from 290 m (Semlitsch and Bodie 2003) to 500 m (Canessa and Parris 2013). We consider the central point of the buffer to be the pond or stream (collection points). The polygons for each type of roof were redesigned for the SIRGAS 2000 reference system, Universal Transverse Mercator (UTM) projection, zone 22S, and we calculated the areas in km².

Functional traits of tadpoles

For the analysis of functional traits diversity, we recorded measurements from one to ten tadpoles of each species. Eighteen morphological traits were evaluated for each species in the study area. The measured functional traits consider the functional characteristics related to the main aspects of the resource acquisition, use of resources and life-history strategies, as follows: tail muscle height (TMH), tail muscle width (TMW), dorsal fin height (DFH), ventral fin height (VFH), body height (BH), body width (BW), body length (BL), oral disk size (ODS), oral disc position (ODP), number of tooth rows (NTR), eye size (ES), internal eye distance (IED), eye position (EP), nostril size (NS), internal nostril distance (IND), nostril position (NP), spiracle length (SL), spiracle width (SW), spiracle position (SP). We also recorded the position in the water column (benthic, nektonic and neustonic), the presence of flagellum (present/absent), and the ontogenetic development stage (Figure 2; Table 2). The classification of the tadpole position in the water column was defined according to the literature (see Queiroz et al. 2015).

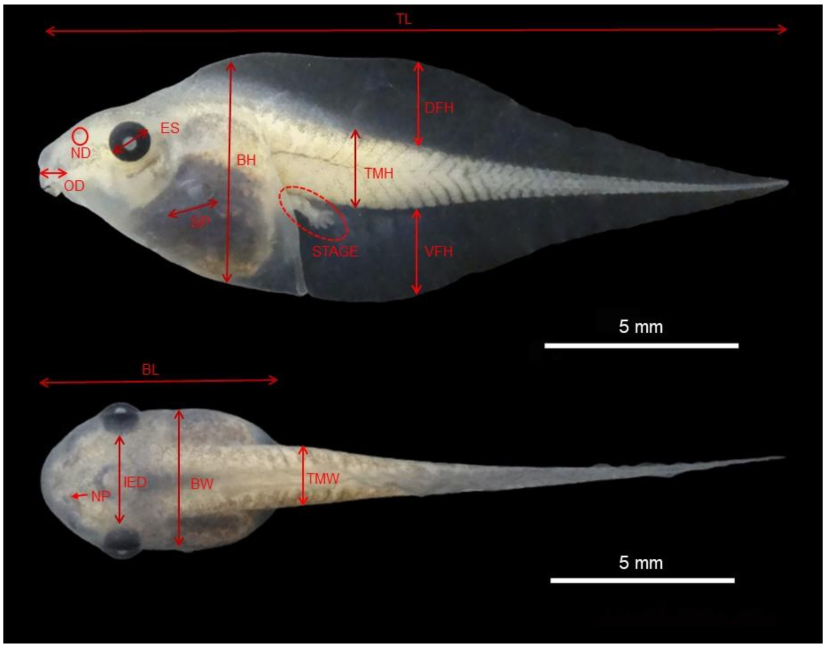


Fig. 2 Morphological metrics evaluated in anuran tadpoles: total length (TL); body length (BL); tail muscle height (TMH); tail muscle width (TMW), dorsal fin height (DFH), ventral fin height (VFH), body height (BH), body width (BW), oral disk size (ODS), oral disc position (ODP), number of tooth rows (NTR), eye size (ES), internal eye distance (IED), eye position (EP), nostril size (NS), internal nostril distance (IND), nostril position (NP), spiracle length (SL), spiracle width (SW), spiracle position (SP). In this picture: Dorsal view and lateral view of *Scinax fuscovarius* tadpoles. (Photographed by Brena da Silva Gonçalves).

Table 2 Functional traits measured from different functional characteristics of tadpoles present in the sampled waterbodies.

Functional trait	Variable	Ecological relevance
Body Height (BH) = body height/total length	Continuous	Associated with tadpole biomass; also determines the position of the species in the water column (1, 2, 3, 4)
Body Length (BL) = Body length/total length	Continuous	
Body Width (BW) = Body width/total length	Continuous	
Tail Muscles Width (TMW) = maximum width of the caudal musculature/body length	Continuous	Related to the species' swimming capacity, the exploration of micro-habitats and, in the case of nektonic tadpoles, the maintenance of balance when they are at rest (1, 2, 4, 5, 6)
Dorsal Fin Height (DFH) = maximum height of dorsal fin/maximum height of the caudal musculature	Continuous	
Ventral Fin Height (VFH) = maximum height of ventral fin/maximum height of the caudal musculature	Continuous	
Internal Eye Distance (IED) = distance between eyes/body length	Continuous	Related to habitat exploration and the species' ability to detect predators (1, 2, 4)
Eye Size (ES) = eye diameter/body length	Continuous	
Number of Tooth Rows (NTR) = Sum of number of front and back rows	Continuous	Directly related to the ability to exploit food resources (1, 2)

Oral Disc Position (ODP) = Anteroventral; ventral	Categoric	
Mouth Size (MS) = mouth size/body length	Continuous	
Presence of flagellum = Present or absent	Binary	Assists in capacity and speed during swimming (5, 6)
Nostrils Diameter (ND) = nostril diameter/body length	Continuous	Directly related to the circulation of water in the body, in addition to assisting the chemical perception of molecules dissolved in water (1, 4)
Nostrils Position (NP) = Absent/laterodorsal/dorsolateral/ anterolateral/lateral/dorsal	Categoric	
Spiracle Length (SL) = Body length/total length	Continuous	The spiracle, together with the operculum, is associated with the regulation and control of respiratory and feeding currents (7, 8)
Spiracle Position (SP) = Ventral/sinistral/posterodorsal /posterior	Categoric	
Spiracle Width (SW) = Body Width/Total Length	Continuous	

REFERENCES (1) Altig and Johnston 1989; (2) Altig and McDiarmid 1999; (3) Buskirk 2009; (4) Jordani et al. 2019; (5) Johnson et al. 2015; (6) Queiroz et al. 2015; (7) Gollman and Gollman 1999; (8) Altig 2006.

Data analysis

Before carrying out the statistical analyses, we transformed our quantitative datasets (functional traits and environmental descriptors) through a natural log transformation; this procedure allows obtaining a normal distribution of the different datasets. Subsequently, we used a combination of the RLQ (Dolédec et al. 1996; Dray et al. 2016) and the fourth-corner analysis (Legendre et al. 1997) for assessing the responses of the set of tadpole functional traits to environmental variation (Dolédec et al.1996; Dray et al. 2014). In a general view, the proposed RLQ approach is an analysis that performs ordering analyses based on the combination of the following data matrices: an environmental matrix by sites (*R*; site x environment), a species-by-sites matrix (*L*; site x species), and a functional-trait by species matrix (*Q*; species x traits). Matrices R and Q are linked by matrix L. The RLQ method is a multivariate technique that performs a double inertia analysis of matrices R and Q, assuming that matrix L is the link between environment and species traits (Dray et al. 2014).

Before the extended RLQ analysis, we ran the extended version of the fourth-corner approach with 9999 permutations to test the correlations between functional traits and environmental descriptors. For this, we applied the null model 6 (which fixes the level of type I error; Dray et al. 2016). To prepare the matrices for the extended RLQ analysis, all matrices were analyzed separately with different ordinations: the species-by-site matrix (L) was analyzed using correspondence analysis (CA); the environmental matrix (R) was analyzed by principal component analysis (PCA); finally, the trait distance matrix (Q) was analyzed by principal coordinate analysis (PCoA). The fourth-corner method also combines matrices R, L and Q into a single matrix describing traits-environment associations (fourth-corner matrix; Legendre et al. 1997). Additionally, this analysis tests the relationship between one trait and one environmental variable at a time, allowing the evaluation of individual trait-environment relationships. RLQ and fourth-corner are complementary methods and their combined use may improve strongly the study of trait-environment associations (Dray et al. 2014). The RLQ analysis and the fourth-corner test were implemented using the packages “spdep” (Bivand et al. 2008) and “ade4” (Dray and Dufour 2007) of R software.

We ran the statistical procedures described above separately for each set of environmental data (i.e., one analysis for local environment data, and another for landscape data) and each type of aquatic system (i.e., ponds and streams). To avoid the inclusion of non-significant environmental descriptors in our models, we performed the analytical procedures described above in two steps. In the first step, we run the analyses containing the full model of environmental descriptors for each dataset (that is, local and landscape descriptors). Subsequently, we performed a new analysis and included only the environmental descriptors that showed significant relationships with the functional traits of the tadpoles (i.e., a selected model). The percentage of co-inertia is available as the link between functional traits and environmental descriptors.

Results

Ponds and streams evaluation

We recorded a total of 22 species with 14 species found in ponds and 19 in streams. The species belong to eight families and 10 genera. Hylidae was the most representative family, with four genera (*Aplastodiscus*, *Boana*, *Dendropsophus* and *Scinax*). The genera *Elachistocleis*, *Proceratophrys* and *Lithobates* occurred only in ponds and the genus *Crossodactylus* only in streams (see Supplementary Information Table S1). The species *Boana curupi* and *Crossodactylus schmidti* are considered forest specialists, while the others are considered habitat generalists (see Santos et al. 2021).

Patterns of trait-environment relationships in ponds

The percentage of co-inertia explained by the two first axes of the fitted RLQ was 75% for the model with local environmental descriptors and 80% for the model with landscape descriptors (Table 3). However, only the model with local environmental descriptors was significant (Std. observed = 3.69, p = 0.001; see Supplementary Information Table S2).

Table 3 RLQ results from ponds, model of local descriptors, and landscapes descriptors at 28 waterbodies in areas of Atlantic Forest in southern Brazil.

Model	**Axis**	**Global RLQ**			**Fitted RLQ**		
		Eigenvalue	%	Cum.%	Eigenvalue	%	Cum.%
Local Descriptors	1	4.44	49.10	49.10	4.10	57.84	57.84
	2	1.91	16.21	65.31	1.22	17.25	75.09
	3	0.96	8.55	73.86	0.79	11.07	86.16
	4	0.82	4.13	77.99	0.47	6.69	92.84
	5	0.50	2.06	80.05	0.36	5.04	97.89
Landscapes Descriptors	1	2.13	40.70	40.70	1.98	55.09	55.09
	2	1.69	32.21	72.91	0.92	25.68	80.76
	3	0.75	14.37	87.28	0.68	18.89	99.65
	4	0.54	10.34	97.62	0.01	0.21	99.87
	5	0.12	2.19	99.80	<0.01	0.13	100.00

Figure 3a-d presents the patterns of trait-environment relationships observed in ponds. The first RLQ axis had the strongest correlation with the local environmental descriptors pond depth and water pH, and with the functional traits related to the body: Spiracle position posterodorsal (SP posterodorsal), internal eyes distance, and eyes position dorsal (IED; EP dorsal), nostrils position dorsal (NP dorsal) dorsal tail height and flagellum absent (DFH; Fl absent). The second RLQ axis had the strongest correlation with trees, water conductivity, and the functional traits related to nostrils position (NP absent) and dorsal tail height (DFH; see Supplementary Information Tables S8-S10). Benthic tadpoles were more associated with high values of water pH, while nektonic tadpoles were more associated with the presence of trees, although no significant relation of this local environmental descriptor was detected for any of the measured functional traits. Finally, deeper water was more associated with the neustonic tadpoles (Figure 3 a-b). The significance tests of trait-environment relationships are presented in Figure 4a and the Supplementary Information Tables S3 and S4.

The first two axes of the RLQ model for the landscape descriptors of ponds accounted for, respectively, 55% and 26% of the variance. Despite this, this model was not statistically significant (Std. observed = 1.447, p = 0.07; Table 3, Figure 3 c-d). However, the fourth-corner test showed significant relationships between some landscape descriptors and functional traits (Figure 4b). We observed a negative relationship between forest and dorsal fin height (DFH); agriculture and spiracle position ventral (SP ventral); pasture and spiracle width (SW); urban and oral disc position ventral (ODP ventral), spiracle position sinistral (SP sinistral), and nostril position laterodorsal (NP laterodorsal); edge distance and body width (BW), oral disc position ventral (ODP ventral), spiracle position sinistral (SP sinistral) and nostril position (NP laterodorsal). Finally, we observed positive relationships between forest and spiracle position ventral (SP ventral), and urban and eye position lateral (EP lateral) (Supplementary Information Tables S5 and S6).

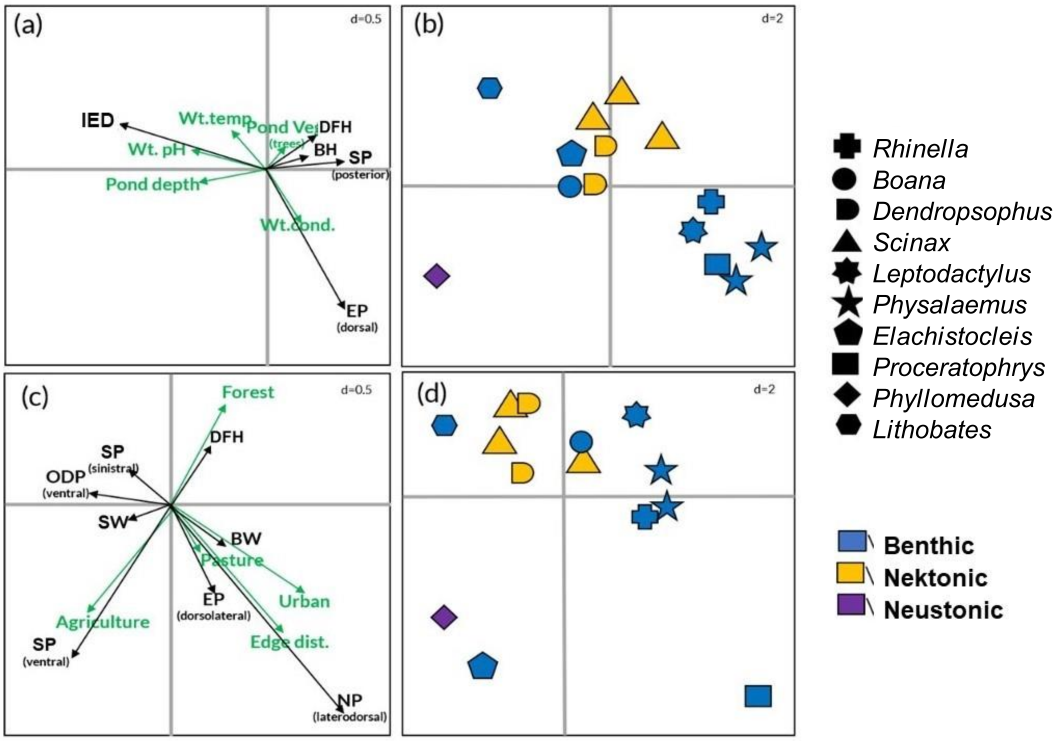


Fig. 3 Ordination of tadpoles' functional traits and ponds' local descriptors (a), anuran genera and tadpoles' ecomophologic guild (b), ordination of functional traits and landscapes descriptors (c) anuran genera and tadpoles' ecomophologic guild (d) result of the RLQ analysis. Genera (b and d) are presented by symbols and tadpole ecomophologic guilds by colors: blue = benthic; yellow = nektonic; purple = neustonic. In (a): IED = Internal eye distance; EP (dorsal) = Eye position dorsal; DFH = Dorsal fin height; BH = Body heigth; SP (posterior) = Spiracle position posterior; Wt.temp. = Water temperature; Wt.pH = Water pH; Wt.cond. = Water conductivity; Pond.veg.(grass) = Pond vegetation grass. In (c): BW = Body width; EP (dorsolateral) = Eye position dorsolateral; SP (ventral) = Spiracle position ventral; SP (sinistral) = Spiracle position sinistral; NP (laterodorsal) = Nostril position laterodorsal; ODP (ventral) = Oral disc position (ventral); SW = Spiracle width; DFH = Dorsal fin height; Edge dist. = Edge distance.

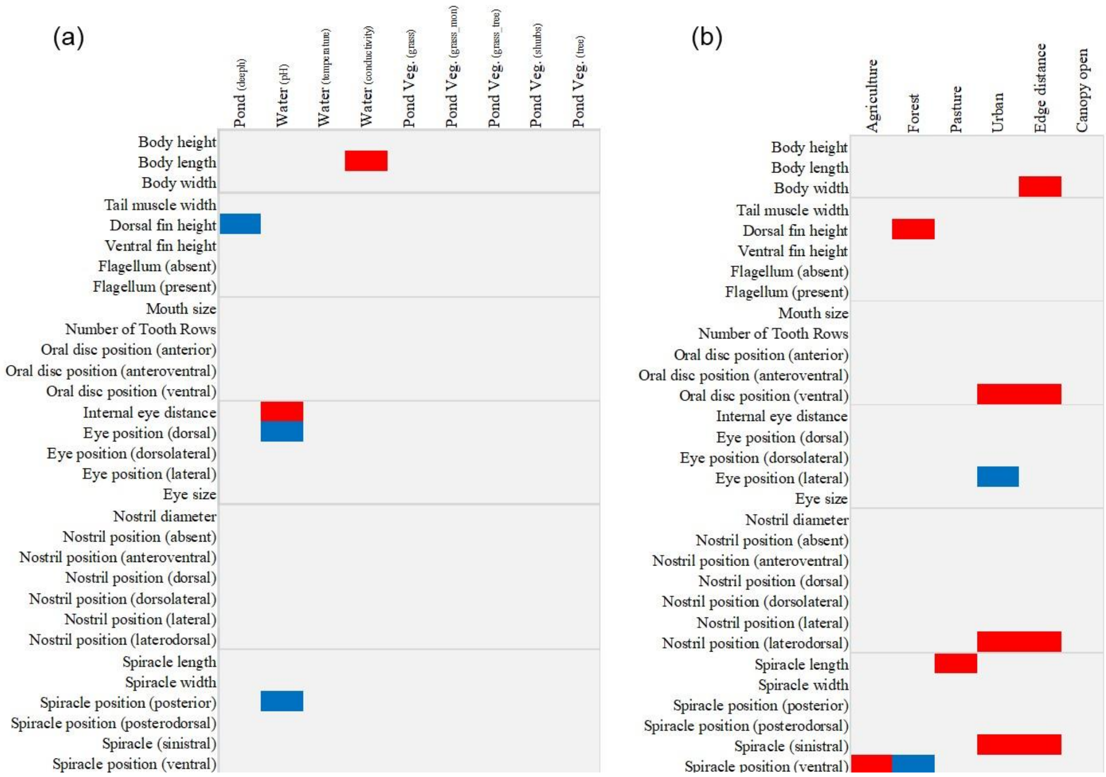


Fig. 4 Schemes representing the associations between the tadpoles' functional traits and ponds' local descriptors (a) and the associations between the tadpoles' functional traits and ponds' landscapes descriptors (b). Colorless cells represent non-significant associations. Positive and negative associations are represented in blue and red, respectively. The lines show the attribute categories of the body, tail, mouth, eyes, nostrils and spiracle. For functional traits, see Table 2. Acronyms of environment attributes in (a): Pond (deeph) = pond water depth; Wt (pH) = Water pH; Wt (temp) = Water temperature; Wt (cond) = Water conductivity; Pond veg. (grass) = Pond vegetation grasses; Pond veg. (grass_mon) = Pond vegetation grass monoculture; Pond veg. (grass_trees) = Pond vegetation grasses and trees; Pond veg. (shrubs) = Pond vegetation shrubs; Pond veg. (trees) = Pond vegetation trees.

Patterns of trait-environment relationships in streams

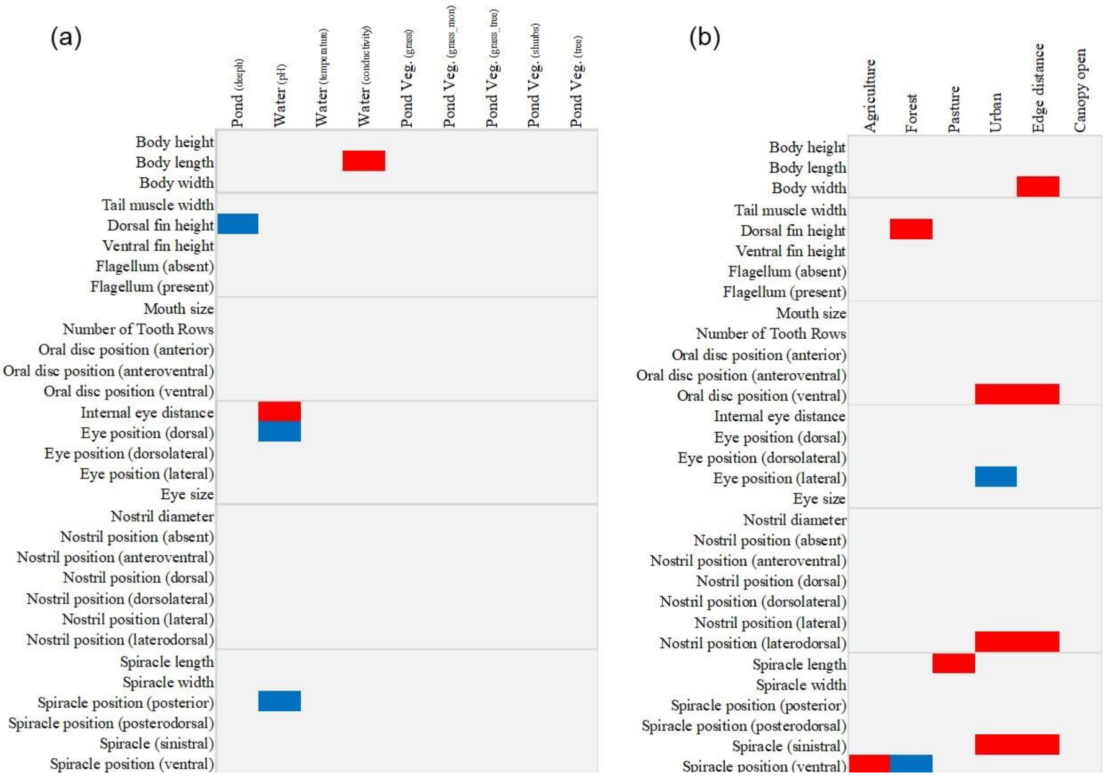
The percentage of co-inertia explained by the two first axes of the fitted RLQ was 73% for the model with local descriptors, and 78% for the model with landscape descriptors (Table 4). Similar to the patterns observed in ponds, only the model with local descriptors was significant (Std. observed = 3.54, p = 0.001; see Supplementary Information Table S7).

Table 4 RLQ results from streams, model of local descriptors, and landscapes descriptors at 28 waterbodies in areas of Atlantic Forest in southern Brazil.

Model	**Axis**	**Global RLQ**			**Fitted RLQ**		
		Eigenvalue	%	Cum.%	Eigenvalue	%	Cum.%
Local Descriptors	1	9.50	46.15	46.15	8.40	48.46	48.46
	2	5.56	27.04	73.19	4.25	24.50	72.96
	3	2.31	11.20	84.39	2.03	11.73	84.69
	4	1.23	5.99	90.39	0.97	5.58	90.27
	5	0.98	4.78	95.17	0.85	4.92	95.19
Landscapes Descriptors	1	4.72	48.43	48.43	2.51	56.84	56.84
	2	2.27	23.30	71.73	0.96	21.67	78.51
	3	1.11	11.43	83.16	0.46	10.37	88.88
	4	0.67	6.93	90.09	0.35	7.81	96.69
	5	0.47	4.79	94.89	0.12	2.71	99.40

Figure 5a-d presents the patterns of trait-environment relationships observed in streams. The first RLQ axis had the strongest correlation with water pH, water temperature, water conductivity, total alkalinity, Alk HCO3, and substrate stream with mud and rocks (mud_rocks; Supplementary Information Tables S5-S7), and with the functional traits related to the body: Body width (BW) and spiracle position posterodorsal (SP posterodorsal), eye position dorsal and lateral (EP dorsal; EP lateral), number of tooth rows and oral disc position anteroventral (NTR; ODP anteroventral), mouth size (MS) and tail with flagellum present (Fl present) and ventral fin height (VFH; Supplementary Information Tables S8 and S9). The second RLQ axis had the strongest correlation with the types of stream vegetation with the presence of trees on the banks and presence of grass and rocks on the substrate and with the functional traits related to the mouth size (MS). Most benthic tadpoles were more associated with the physicochemical descriptors of water (pH, temperature) and with trees, while the second group of benthic species (consisting of the genera *Aplastodiscus*, *Boana,* and *Lithobates*) was more associated with the physicochemical descriptors of water NO3, Alk HCO3, total alkalinity and conductivity. Nektonic tadpoles were more associated with stream area and water transparency. Finally, the neustonic tadpoles were more associated with the presence of aquatic vegetation on the stream substrate (Figure 5a-b). The significance tests of trait-environment relationships are presented in Figure 6a.

The two first axes of the RLQ model to the landscape descriptors of streams accounted for, respectively, 57% and 22% of the variance. Despite this, this model was not statistically significant (Std. observed = 1.33, p = 0.09; Table 4; Supplementary Information Tables S10 and S11). However, the fourth-corner test showed significant relationships between canopy opening and the functional trait nostril position dorsal (NP dorsal) (Figure 6b; Supplementary Information Table S11).



	Pond (deeph)	Water (pH)	Water (temperature)	Water (conductivity)	Pond Veg. (grass)	Pond Veg. (grass_mon)	Pond Veg. (grass_tree)	Pond Veg. (shubs)	Pond Veg. (tree)
Body height									
Body length				🟥					
Body width									
Tail muscle width									
Dorsal fin height	🟦								
Ventral fin height									
Flagellum (absent)									
Flagellum (present)									
Mouth size									
Number of Tooth Rows									
Oral disc position (anterior)									
Oral disc position (anteroventral)									
Oral disc position (ventral)									
Internal eye distance		🟥							
Eye position (dorsal)		🟦							
Eye position (dorsolateral)									
Eye position (lateral)									
Eye size									
Nostril diameter									
Nostril position (absent)									
Nostril position (anteroventral)									
Nostril position (dorsal)									
Nostril position (dorsolateral)									
Nostril position (lateral)									
Nostril position (laterodorsal)									
Spiracle length									
Spiracle width									
Spiracle position (posterior)		🟦							
Spiracle position (posterodorsal)									
Spiracle (sinistral)									
Spiracle position (ventral)									

	Agriculture	Forest	Pasture	Urban	Edge distance	Canopy open
Body height						
Body length						
Body width					🟥	
Tail muscle width						
Dorsal fin height		🟥				
Ventral fin height						
Flagellum (absent)						
Flagellum (present)						
Mouth size						
Number of Tooth Rows						
Oral disc position (anterior)						
Oral disc position (anteroventral)						
Oral disc position (ventral)				🟥	🟥	
Internal eye distance						
Eye position (dorsal)						
Eye position (dorsolateral)						
Eye position (lateral)				🟦		
Eye size						
Nostril diameter						
Nostril position (absent)						
Nostril position (anteroventral)						
Nostril position (dorsal)						
Nostril position (dorsolateral)						
Nostril position (lateral)						
Nostril position (laterodorsal)				🟥	🟥	
Spiracle length			🟥			
Spiracle width						
Spiracle position (posterior)						
Spiracle position (posterodorsal)						
Spiracle (sinistral)				🟥	🟥	
Spiracle position (ventral)	🟥	🟦				

Fig. 5 Ordination of tadpoles' functional traits and stream' local descriptors (a), anuran genera and tadpoles' ecomophologic guild (b), ordination of functional traits and landscapes descriptors (c) anuran genera and tadpoles' ecomophologic guild (d) result of the RLQ analysis. Genera (b and d) are presented by symbols and tadpole ecomophologic guilds by colors: blue = benthic; yellow = nektonic; purple = neustonic. In (a): BH = Body height; BL = Body length; BW = Body width; DFH = Dorsal fin height; IED = Internal eye distance; ES = Eye size; EP (dorsal) = Eye position dorsal; Fl (pres.) Flagellum presence; ND = Nostril diameter; MS = Mouth size; SL = Spiracle length; SP (posterior) = Spiracle position posterior; SP (posterodorsal) = Spiracle position postererodorsal; SW = Spiracle width; Wt.temp. = Water temperature; Wt.pH = Water pH; Wt.cond. = Water conductivity; Pond veg. (Grass) = Pond vegetation grass. In (c): NP (dorsal) = Nostril position dorsal.

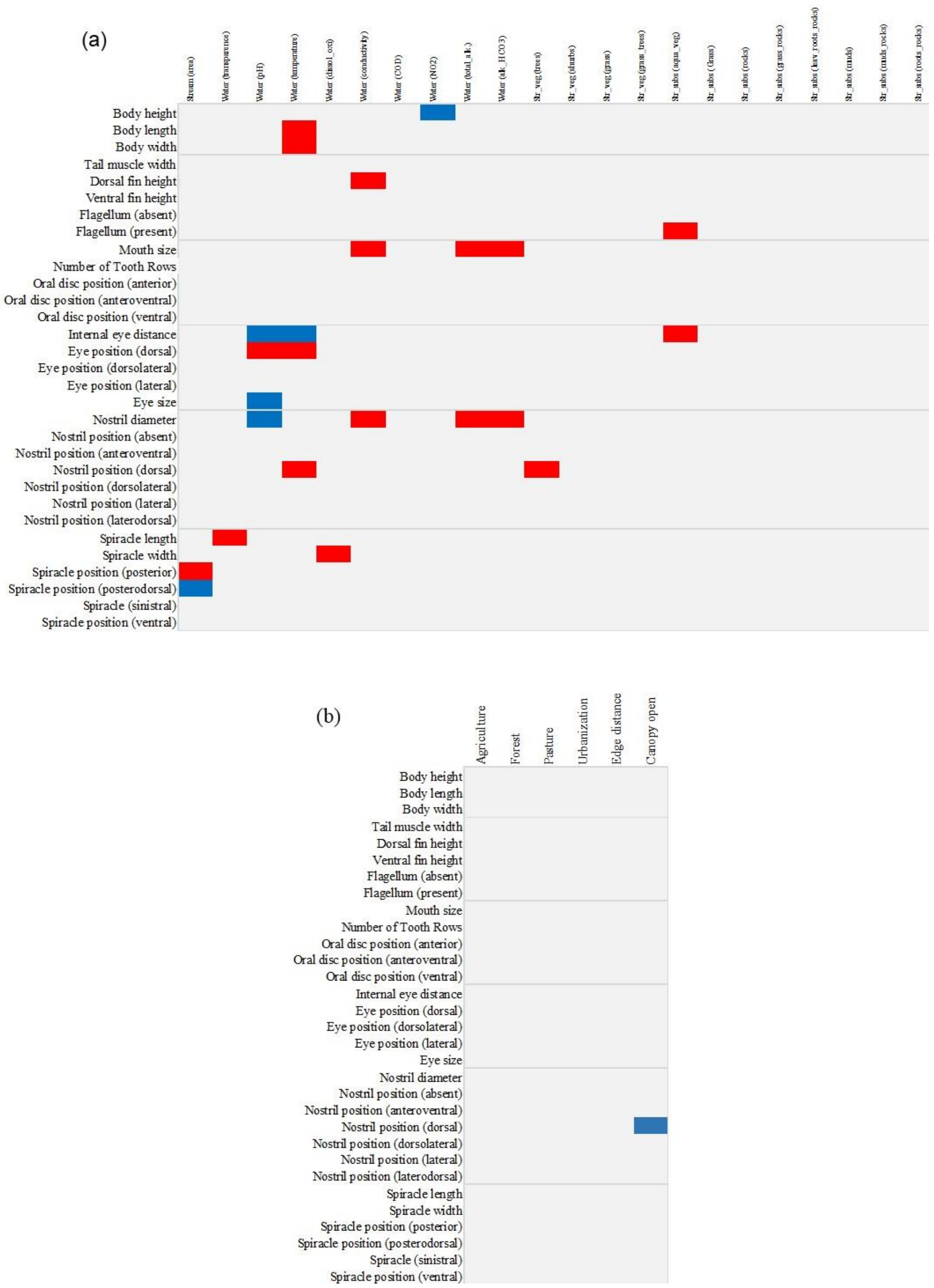


Fig. 6 Schemes representing the associations between the tadpoles functional traits and stream local descriptors (a) and the associations between the tadpoles functional traits and stream landscapes descriptors (b). Colorless cells represent non-significant associations. Positive and negative associations are represented in blue and red, respectively. The lines show the attribute categories of the body, tail, mouth, eyes, nostrils and spiracle. For functional traits, see Table 2. Abbreviations of environment attributes, in (a): Stream (area) = Stream area; Wt (transparency) = Water transparency; Wt (pH) = Water pH; Wt (temperature) = Water temperature; Wt (dissol_oxi) = Water dissolved oxygen; Wt (cond) = Water conductivity; Wt (COD) = Water chemical oxygen demand; Wt (NO2-) = Water nitrite; Wt (total_alk) = Water total alkalinity; Wt

(Alk_HCO3) = Bicarbonate alkalinity; Str_Veg (trees) = Stream vegetation trees; Str_Veg (shrubs) = Stream vegetation shrubs; Str_Veg (grass) = Stream vegetation grasses; Str_Veg (grass_trees) = Stream vegetation grasses and trees; Str_subs (aqua_veg) = Stream substrate with aquatic vegetation; Str_subs (grass) = Stream substrate with grasses; Str_subs (rocks) = Stream substrate with rocks; Str_subs (grass_rocks) = Stream substrate with grasses and rocks; Str_subs (leave_roots_rocks) = Stream substrate with leaves, roots and rocks; Str_subs (mud) = Stream substrate with mud; Str_subs (mud_rocks) = Stream substrate with mud and rocks; Str_subs (roots_rocks) = Stream substrate with roots and rocks.

Discussion

Our results pointed out that the local habitat variables are more relevant than the landscape variables to define functional traits in tadpole communities in ponds and streams. Based on the differences between ponds and streams regarding abiotic (Smith et al. 2002; Fairchild and Velinsky 2006; Hoeinghaus et al. 2007) and biotic components (Schriever and Lytle 2016; Jordani et al. 2017) we predicted that communities were exposed to different selective pressures that will affect the tadpole traits differently. This idea is supported by previous studies in which environmental characteristics, including properties of the waterbody, are important drivers for variations in tadpole morphology (Sun et al. 2021). Furthermore, we observed differences even in the type of association that each environmental descriptor caused on the functional trait of tadpoles between these two waterbody types. It is interesting to notice that, despite the evident differences in terms of functional traits, the species composition in these two systems was similar. The relatively large number of shared species between ponds and streams suggests that the effects of environmental filters over the communities are less prone to be detected based on a taxonomical evaluation (species composition). This reinforces the relevance of a functional approach in a search for ecological differences between communities. In addition, the effect of environmental components on individual variations within the same species would reveal another source of variation in response to habitat, which is another aspect worth being investigated (Jordani et al. 2019). The exclusivity of some species had a strong relationship with reproductive mode, i.e., species that are exclusive to stream communities lay their eggs only in this type of habitat (*Boana curupi* and *Crossodactylus schmidti*; Carrizo 1991; Caldart et al. 2014), and the same can be said for species that occur mainly in ponds (*Elachistocleis*, *Proceratophrys* and *Lithobates*) (Rodrigues et al. 2003; Both 2012).

In ponds, water depth was an environmental variable associated with a large number of functional traits. It was, for example, positively associated with the dorsal height of the tail fin, which would affect the swimming of tadpoles. Since fin height contributes to displacement in the water column (Johnson et al. 2015; Jordani et al. 2019), this would be an important factor, especially for nektonic species (e.g., *Dendropsophus* and *Scinax*), as it increases their ability to exploit different resources in the water column (Marques and Nomura 2018). The relationship between depth and functional traits deserves attention since the functional diversity of tadpoles tends to increase in medium depths, suggesting that it is surrounded by a complex set of ecological interactions (Queiroz et al. 2015). However, attributing functional traits to a single environmental factor means approaching the ecological and adaptive processes that may be acting on this process in a simplified manner. Alternatively, we can associate pond depth to the pond's cycle. In the case of shallower ponds, depth reflects the pond's longevity (hydroperiod), which defines the possibility of a community occupying a place (Jordani et al. 2017; Meyer et al. 2020), giving the tadpoles environmental clues for the timing of their metamorphosis. The speed at which temporary ponds dry out interferes with the speed that is needed for metamorphosis and the size of the newly metamorphosed (Wellborn et al. 1996; Babbitt et al. 2003; Johnson et al. 2015).

The fact that depth was an important component only in ponds may be related to the low stability and predictability of the ponds that were sampled in our study area. Hydroperiod also affects the processes of predation and/or competition (Wellborn et al. 1996; Werner et al. 2007; Simpkins et al. 2013; Melo et al. 2018). This can justify the association that we recorded between water depth and morphology of the eyes (predator detection), tail (mobility, escape velocity) and body size (susceptibility to predation).

However, associating morphology with specific environmental factors is a complex and risky task (Lopes et al. 2020). Nevertheless, some generalizations can be made. The position of the eyes, for example, would be related to habitat evaluation, and the height of the tail's dorsal fin and filament are related to the locomotion capacity (Altig and Johnston 1989; Altig and McDiarmid 1999; Johnson et al. 2015; Queiroz et al. 2015; Jordani et al. 2019), while nostrils would be related to physiological activities of osmoregulation (Altig and Johnston 1989; Jordani et al. 2019), and the morphology of the mouth apparatus would be associated with feeding (Altig and Johnston 1989; Altig and

McDiarmid 1999). Thus, it is possible to expect morphological changes to result from processes of physiological compensations for a certain environmental stressor. We could, therefore, speculate about the morphology-physiology relationship and changes in functional traits. There are indications, for example, that an increase in tail height may be a response to predation pressure (McCollum and Leimberger 1997; Relyea 2003; Relyea and Hoverman 2003), and the effects of the predation risk on microhabitat selection are well known in aquatic organisms. Vegetation, for example, enhances the use of microhabitats with greater refuge availability (Diaz-Paniagua 1987; Kopp et al. 2006), interfering in the predation process. Other evaluated local variables, such as pH, water conductivity and presence of arboreal vegetation were associated with functional traits of the nostrils, spiracle, eyes and tail morphology. Physicochemical components of water may affect the physiology and development of tadpoles, which reflects in compensation mechanisms. Low pH values may affect tadpole growth (Pierce 1985; Farquharson et al. 2016; Meyer et al. 2020). The traits of nostrils and spiracle are related to the tadpoles' respiratory and regulatory physiology, water intake and flow into the body (Gollman and Gollman 1999; Altig 2006), which has a strong connection to the physicochemical properties of water. Water conductivity, in turn, is related to the susceptibility to diseases (e.g., bacteria and the fungus *Batrachochytrium denbrobatidis*; Carey 1993; Klaver et al. 2013).

It is interesting to notice that the physical and chemical descriptors of water revealed to be more relevant for stream communities than for pond communities. In streams, functional traits were associated with a larger set of descriptors, such as pH, temperature, conductivity, total alkalinity, Alk and HCO3. Besides the water descriptors, the most relevant components in streams were linked to the substrate and the characteristics of the margin, such as vegetation type (tree). While the water parameters are more connected to physiological processes, the microhabitat elements may be more related to foraging and shelter provision (Williams et al. 2008). This hypothesis is reinforced by the fact that this set of descriptors showed a strong association with functional traits that could be related to predation susceptibility (body size and morphology of fins and the tail), as well as to habitat exploration and acquisition of food resources (position of the eyes, mouth and oral disc) (Altig and Johnston 1989; Altig and McDiarmid 1999; Jordani et al. 2019).

Another interesting point is that we found species groups linked to their guilds, but the descriptors that are more associated with them are habitat-specific. Previous studies pointed out that microhabitat environmental variables were the primary drivers of the taxonomic variation and functional diversity in tadpole communities (see Sun et al. 2021). Variations in the microhabitat would generate drastic changes in the availability of food and shelter for tadpoles (Melo et al. 2017). In streams, most benthic species are more associated with descriptors of water (pH, temperature, NO3, HCO3, total alkalinity and conductivity) and with trees. On the other hand, in ponds, benthic species were only associated with pH. In streams, nektonic species were more associated with stream area and greater water transparency, while in ponds they were associated only with the presence of trees. Neustonic tadpoles, in turn, were more associated with the presence of aquatic vegetation on the stream substrate in streams and with depth in ponds. We found that stream-associated guilds were related to a larger set of microhabitat variables than pond-related guilds. Thus, our data reinforce that, in each aquatic system, species have particular patterns of microhabitat use that depend on intrinsic aspects of the type of waterbody (Melo et al. 2018).

Although we cannot associate these differences with elements of their life history, the fact that there is a relationship between guilds and different habitat parameters and respective functional traits is extremely relevant. Some derivations can be listed, although speculatively. Temperature, for example, is one of the main local variables that influence the physical, chemical and biological processes of streams (Caissie 2006). Studies relating tadpole growth to temperature show that the latter affects tadpole development (see Browne and Edwards 2003), with growth speeding up under ideal temperatures for tadpole growth (Maciel and Juncá 2009). Because permanent ponds are lentic systems, they are expected to be more stable environments regarding water parameters than streams. Depth would be a variable defining the stability level of this system: the deeper, the more stable. However, the surrounding environment of ponds and streams has a great potential to change the physical and chemical characteristics of water (Sipaúba-Tavares et al. 2007; Mansano et al. 2012, 2014). The vegetation at the margins of streams and ponds acts as an important resource and filter of organic matter for aquatic environments (Lecerf et al. 2005), affecting energy flow and the whole local trophic web (Antoniazzi et al. 2020). Our results indicated a possible association in the functional traits related to physicochemical characteristics of the water and microhabitat used by tadpoles in the waterbodies. These association, highlighted by differences in functional traits between ponds and streams, indicates an ecological response in the face of different environmental conditions. These responses can be seen as an important survival tool in systems that are susceptible to fast changes in their conditions. Rain, sediment input and the arrival of predators are important elements in the stability of the habitat of tadpoles. Since tadpoles have a short time to complete their metamorphosis, the possibility of quick responses in the face of dynamic and somewhat unpredictable environmental conditions would be an important tool for the reproductive success of anurans.

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Authors' contributions R.C.S., D.A.D., D.B., R.K.F., E.M.L. and A.M.T designed the study and wrote the paper. R.C.S. and D.B. collected and processed the data. D.A.D. conducted the statistical analyses.

Statements and Declarations

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References

Afonso LG, Eterovick PC (2007) Spatial and temporal distribution of breeding anurans in streams in southeastern Brazil. J Nat Hist. 41:949–963. https://doi.org/10.1080/00222930701311680

Altig R, Johnston GF (1989) Guilds of anuran larvae: Relationships among modes, morphologies, and habitats. Herpetol Monogr 3:81–109

Altig R, McDiarmid RW (1999) Body plan: Development and Morphology. In Altig R, McDiarmid RW (ed) Tadpoles: The Biology of Anuran Larvae. University of Chicago Press, Chicago, pp. 24-51

Altig R (2006) Tadpoles evolved and frogs are the default. Herpetologica 62:1–10

Alvares CA, Stape JL, Sentelhas PC et al (2013) Köppen's climate classification map for Brazil. Meteorol Zeitschrift 22:711–728. https://doi.org/10.1127/0941-2948/2013/0507

Antoniazzi CE, López JA, Lorenzón RE et al (2020) Trophic ecology of tadpoles in floodplain wetlands: combining gut contents, selectivity, and stable isotopes to study feeding segregation of syntopic species. Hydrobiologia 847:3013–3024. https://doi.org/10.1007/s10750-020-04303-0

APHA (1998) Standard methods for the examination of water and wastewater. 20th edition. American Public Health Association, Washington

Arruda Almeida BD, Green AJ, Sebastián-González E et al (2018) Comparing species richness, functional diversity and functional composition of waterbird communities along environmental gradients in the neotropics. PLoS One 13:e0200959. https://doi.org/10.1371/ journal.pone.0200959

Babbitt KJ, Baber MJ, Tarr TL (2003) Patterns of larval amphibian distribution along a wetland hydroperiod gradient. Can J Zool 81:1539–1552

Bivand RS, Pebesma EJ, Gómez-Rubio V (2008) Applied Spatial Data Analysis with R. Springer, New York, London

Bolzan AMR, Saccol SA, Santos TGD (2016) Composition and diversity of anurans in the largest conservation unit in Pampa biome, Brazil. Biota Neotrop 16:e20150113. https://doi.org/10.1590/1676-0611-BN-2015-0113

Borges FF, Stéfani MV, Amaral LA (2014) Quality of the effluents of bullfrog tadpole ponds. Bol Inst Pesca 40:409–417

Both C (2012) Invasão de *Lithobates catesbeianus* na mata atlântica sul do brasil: relações com espaço, ambiente e anfíbios nativos. Thesis, Pontifícia Universidade Católica do Rio Grande do Sul, Brazil

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

Browne R, Edwards D (2003) The effect of temperature on the growth and development of the endangered green and golden bell frog (*Litoria aurea*). J Therm Biol 28:295–299

Buskirk JV (2009) Natural variation in morphology of larval amphibians: Phenotypic plasticity in nature? Ecol Monogr 79:681–705

Caissie D (2006) The thermal regime of rivers: a review. Freshw Biol 51:1389–1406

Caldart VM, Iop S, Cechin SZ (2014) Social interactions in a neotropical stream frog reveal a complex repertoire of visual signals and the use of multimodal communication. Behaviour 151:719–739. https://doi.org/10.1163/1568539X-00003165

Canessa S, Parris KM (2013) Multi-scale, direct and indirect effects of the urban stream syndrome on amphibian communities in streams. PLoS One 8:e70262. https://doi.org/10.1371/journal.pone.0070262

Carey C (1993) Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. Conserv Biol 7:355–362

Carrizo G R (1991) Sobre los hílidos de Misiones, Argentina, con la descripción de una nueva especie, *Hyla caingua* n. sp. (Anura, Hylidae). Cuad herpetol 5:32–39

Carvalho RA, Tejerina-Garro FL (2015) The influence of environmental variables on the functional structure of headwater stream fish assemblages: a study of two tropical basins in Central Brazil. Neotrop Ichthyol 13:349–360. https://doi.org/10.1590/1982-0224-20130148

Castaneda AJ (2014) The effects of water and habitat quality on amphibian assemblages in agricultural ditches. Master’s Thesis. Purdue University Fort Wayne

CONCEA - Conselho Nacional para o Controle de Experimentação Animal (2018) Normative Resolution nº 37. National Council for Animal Experimentation Euthanasia Practice Directive. Official Diary, Brasilia

Cornwell WK, Schwilk LD, Ackerly DD (2006) A trait-based test for habitat filtering: convex hull volume. Ecology 87(6):1465-71. doi: 10.1890/0012-9658(2006)87[1465:attfhf]2.0.co;2. PMID: 16869422

Dalmolin DA, Tozetti AM, Pereira MJR (2020) Turnover or intraspecific trait variation: explaining functional variability in a neotropical anuran metacommunity. Aquat Sci 82:1–15. http://doi.org/10.1007/s00027-020-00736-w

Diaz S, Cabido M (2001) Vive la difference: plant functional diversity matters to ecosystem processes. Trends Ecol Evol 16:646–655

Diaz-Paniagua C (1987) Tadpole distribution in relation to vegetal heterogeneity in temporary ponds. Herpetol J 1:167–169

Dolédec S, Chessel D, Ter Braak CJF et al (1996) Matching species traits to environmental variables: A new three-table ordination method. Environ Ecol Stat 3:143–166

Dray S, Blanchet G, Borcard D et al (2016) Adespatial: multivariate multiscale spatial analysis. R package version. https://cran.r-project.org/web/packages/adespatial/

Dray S, Choler P, Dolédec S et al (2014) Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. Ecology 95:14–21. https://doi.org/10.1890/13-0196.1

Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. J Stat Softw 22:1–20

Duellman WE, Trueb L (1994) Biology of Amphibians. Johns Hopkins University Press, Baltimore

Eterovick PC, Barata IM (2006) Distribution of tadpoles within and among Brazilian streams: the influence of predators, habitat size and heterogeneity. Herpetologica 62:365–377

Fairchild GW, Velinsky DJ (2006) Effects of small ponds on stream water chemistry. Lake Reserv Manag 22:321–330. https://doi.org/10.1080/07438140609354366

Farquharson C, Wepener V, Smit NJ (2016) Acute and chronic effects of acidic pH on four subtropical frog species. Water SA 42:52. https://doi.org/10.4314/wsa.v42i1.07

Frazer GW, Canham CD, Lertzman KP (1999) Manual do Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York

Gollmann B, Gollmann G (1999) Plasticity of operculum development in *Bombina orientalis* (Amphibia, Anura). Folia Zool 48:233–236

Goswami M, Bhattacharyya P, Mukherjee I et al (2017) Functional diversity: An important measure of ecosystem functioning. Adv Microbiol 7:82–93. https://doi.org/10.4236/aim.2017.71007

Gonçalves DDS (2014) Diversidade e chave de identificação para girinos ocorrentes em áreas de floresta com araucária. Ph.D. Dissertation, Universidade Federal do Paraná

Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecol Lett 4:379–391

Haddad NM, Brudvig A, Clobert J et al (2015) Habitat fragmentation and its lasting impact on Earth’s ecosystems. Sci Adv 1:e1500052. https://doi.org/10.1126/sciadv.1500052

Heyer WR (1976) Studies in larval amphibian habitat partitioning. Smithsonian Contributions to Zoology. Smithsonian Institution Press, Washington DC. https://doi.org/10.5479/si.00810282.242

Hoeinghaus DJ, Winemiller KO, Birnbaum JS (2007) Local and regional determinants of stream fish assemblage structure: inferences based on taxonomic vs. functional groups. J Biogeogr 34:324–338. https://doi.org/10.1111/j.1365-2699.2006.01587.x

Hooper DU, Chapin IIIFS, Ewel JJ et al (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol Monogr 75:3–35

IAP – Instituto Ambiental do Paraná (2004) Plano de Manejo do Parque Estadual de Vila Velha. Curitiba. http://www.iap.pr.gov.br/pagina-1255.html

Johnson JB, Saenz D, Adams CK et al (2015) Naturally occurring variation in tadpole morphology and performance linked to predator regime. Ecol Evol 5:2991–3002. https://dx.doi.org/10.1002/ece3.1538

Jordani MX, Melo LSO, Queiroz CS et al (2017) Tadpole community structure in lentic and lotic habitats: richness and diversity in the Atlantic Rainforest lowland. Herpetol J 27:299-306

Jordani MX, Mouquet N, Casatti L et al (2019) Intraspecific and interspecific trait variability in tadpole meta-communities from the Brazilian Atlantic rainforest. Ecol Evol 9:4025-4037. https://doi. org/10.1002/ece3.5031

Klaver RW, Peterson CR, Patla DA (2013) Influence of water conductivity on amphibian occupancy in the greater yellowstone ecosystem. West N Am Nat 73:184–197. https://doi.org/10.3398/064.073.0208

Knauth DS, Moreira LFB, Maltchik L (2018) Partitioning tadpole beta diversity in highland ponds with different hydroperiods. Freshw Sci 37:380–388. https://doi.org/10.1086/697926

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

Lecerf A, Dobson M, Dang C K et al (2005) Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. Oecologia 146:432–442

Lescano J, Miloch D, Leynaud G (2018) Functional traits reveal environmental constraints on amphibian community assembly in a subtropical dry forest. Austral Ecol 43:623-634. https://doi.org/10.1111/aec.12607

Lima SL, Casali AP, Agostinho CA (2003) Desempenho zootécnico e percentual de consumo de alimento de rã-touro (*Rana catesbeiana*) na fase de recria (pós-metamorfose) do sistema anfigranja. Rev Bras Zootec 32:505–511

Lipinski VM, Schuch AP, Santos TG (2020) Changes on anuran tadpole functional diversity along an environmental gradient at the southern most Atlantic Rainforest remnant. Asian Herpetol Res 11:63–70. https://doi.org10.16373/j.cnki.ahr.190039

Liu X, Swenson NG, Lin D et al (2016) Linking individual-level functional traits to tree growth in a subtropical forest. Ecology 97:2396-2405. https://doi.org/10.1002/ecy.1445

Lopes GN, Serra RTA, Piorski NM et al (2020) Pond characteristics influence the intraspecific variation in the morphometry of the tadpoles of two species of Dendropsophus (Anura: Hylidae) from the Cerrado savanna of northeastern Brazil. An Acad Bras Cienc 92:e20181171. https://doi.org/10.1590/0001-3765202020181171

Machado IF, Maltchik L (2007) Check-list of diversity of anurans in Rio Grande do Sul (Brazil) and a classification propose for larval forms. Neotrop Biol Conserv 2:101–116

Maciel TA, Juncá FA (2009) Effects of temperature and volume of water on the growth and development of tadpoles of *Pleurodema diplolister* and *Rhinella granulosa* (Amphibia: Anura). Zoologia 26:413–418

Maltchik L, Rolon AS, Stenert C et al (2011) Can rice field channels contribute to biodiversity conservation in Southern Brazilian wetlands? Rev Biol Trop 59:1895–1914

Mansano CFM, Stéfani MV, Pereira MM et al (2012) Non-linear growth models for bullfrog tadpoles. Cienc Agrotec 36:454–462. https://doi.org/10.1590/S1413-70542012000400010

Mansano CFM, Stéfani MV, Pereira MM et al (2014) Morphometric growth characteristics and body composition of bullfrog tadpoles in captivity. Semina: Ciências Agrárias 35: 2817–2830

Mansano CFM, Vanzela LS, Américo-Pinheiro JHP et al (2018) Importance of optimum water quality indices in successful frog culture practices. In: Gökçe D (ed) Limnology: Some new aspects of inland water ecology. IntechOpen, London, pp 133-154

Marques N, Nomura F (2018) Environmental and spatial factors affect the composition and morphology of tadpole assemblages. Can J Zool 96:1130-1136

Marques NCS, Rattis L, Nomura F (2018) Local environmental conditions affecting anuran tadpoles' microhabitat choice and morphological adaptation. Mar Freshw Res 70:395–401. https://doi.org/10.1071/mf18106

Mazerolle MJ, Desrochers A, Rochefort L (2005) Landscape characteristics influence pond occupancy by frogs after accounting for detectability. Ecol Appl 15:824–834. doi:10.1890/04-0502

McCollum SA, Leimberger JD (1997) Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. Oecologia 109:615–621

Meyer EA, Franklin CE, Cramp RL (2020) Physiological and morphological correlates of extreme acid tolerance in larvae of the acidophilic amphibian *Litoria cooloolensis*. J Comp Physiol B 191:159–171. https://doi.org/10.1007/s00360-020-01316-y

Melo L, Gonçalves-Souza T, Garey M, Rossa-Feres D (2017) Tadpole species richness within lentic and lotic microhabitats: An interactive influence of environmental and spatial factors. Herpetol J 27:339–345

Melo LSO, Garey MV, Rossa-Feres DC (2018) Looking for a place: how are tadpoles distributed within tropical ponds and streams? Herpetol Notes 11:379–386

Menezes BS, Martins FR, Dantas Carvalho EC, Souza BC, Silveira AP, Loiola MIB, Araújo, FS (2020) Assembly rules in a resource gradient: Competition and abiotic filtering determine the structuring of plant communities in stressful environments. PLoS ONE 15:e0230097. https://doi.org/10.1371/journal.pone.0230097

Mogali SM, Shangabhag BA, Saidapur SK (2021) Expression of metamorphic traits in four species of anurans (from south India) reared in the laboratory. Herpetol Notes 14:617–623

Oliveira M, Aver G, Moreira LFB, Colombo P, Tozetti AM (2016) Daily Movement and Microhabitat Use by the Blacksmith Treefrog *Hypsiboas faber* (Anura: Hylidae) during the Breeding Season in a Subtemperate Forest of Southern Brazil. South American Journal of Herpetology, 11: 89-97.

Petchey OL, Hector A, Gaston KJ (2004) How do different measures of functional diversity perform? Ecology 85:847–857. https://doi.org/10.1890/03-0226

Petchey OL, O’Gorman EJ, Flynn DF (2009) A functional guide to functional diversity measures. In: Naeem S, Bunker DE, Hector A, Loreau M, Perrings C (ed) Biodiversity, ecosystem functioning, and human wellbeing an ecological and economic perspective. Oxford Scholarship Online, Oxford, pp 49-59

Pianka ER (1973) The structure of lizard communities. Annu Rev Ecol Evol Syst 4:53–74

Pierce BA (1985) Acid tolerance in amphibians. BioScience 35:239–243. http://dx.doi.org/10.2307/1310132

Pillar VP, Vélez E (2010) Extinção dos Campos Sulinos em unidades de conservação: um fenômeno natural ou um problema ético? Nat Conserv 8:84–86

Pulsford SA, Barton PS, Driscoll DA et al (2019) Interactive effects of land use, grazing and environment on frogs in an agricultural landscape. Agric Ecosyst Environ 281:25–34. doi:10.1016/j.agee.2019.05.003

Queiroz CS, Silva FR, Rossa-Feres DC (2015) The relationship between pond habitat depth and functional tadpole diversity in an agricultural landscape. R Soc Open Sci 2:150165. https://doi.org/10.1098/rsos.150165

Relyea RA (2003) How prey respond to combined predators: a review and an empirical test. Ecology 84:1827–1839

Relyea RA, Hoverman JT (2003) The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. Oecologia 134:596–604

Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM (2009) The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biol Conserv 142:1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021

Rodrigues DJ, Lopes FS, Uetanabaro M (2003) Padrão Reprodutivo de *Elachistocleis bicolor* (Anura, Microhylidae) na Serra da Bodoquena, Mato Grosso do Sul, Brasil. Iheringia Série Zoologica 93:365–371

Santa Catarina (1986) Gabinete de Planejamento e Coordenação Geral. Atlas de Santa Catarina. Aerofoto Cruzeiro, Rio de Janeiro

Santos TGD, Rossa-Feres DDC, Casatti L (2007) Diversidade e distribuição espaço-temporal de anuros em região com pronunciada estação seca no sudeste do Brasil. Iheringia. Série Zoologia 97:37–49

Santos MB, Madalozzo B, Tozetti AM et al (2020) Climatic dependence in the daily and seasonal calling activity of anurans from coastal wetlands of southernmost Brazil. J Nat Hist 54:2037–2052. https://doi.org/10.1080/00222933.2020.1833998

Santos RC, Damolin DA, Brum, D et al (2021) Association Between Land Use and Composition of Amphibian Species in Temperate Brazilian Forest Habitats. Herpetol Conserv Biol 16:612–623

Schiesari L (2006) Pond canopy cover: a resource gradient for anuran larvae. Freshw Biol 51:412–423. doi:10.1111/j.1365-2427.2005.01497.x

Schriever TA, Lytle DA (2016) Convergent diversity and trait composition in temporary streams and ponds. Ecosphere 7:e01350. https://doi.org/10.1002/ecs2.1350

Sherratt E, Vidal-García M, Anstis M et al (2017) Adult frogs and tadpoles have different macroevolutionary patterns across the Australian continent. Nat Ecol Evol 1(9):1385–1391. doi:10.1038/s41559-017-0268-6

Sherratt E, Anstis M, Keogh JS (2018) Ecomorphological diversity of Australian tadpoles. Nat Ecol Evol 8(24):12929–12939

Semlitsch RD, Bodie JR (2003) Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. Conserv Biol 17:1219–1228

Simpkins CA, Shuker JD, Lollback GW et al (2013) Environmental variables associated with the distribution and occupancy of habitat specialist tadpoles in naturally acidic, oligotrophic waterbodies. Austral Ecol 39:95–105. https://doi.org/10.1111/aec.12048

Sipaúba-Tavares LH, Guariglia CST, Braga FMS (2007) Effects of rainfall on water quality in six sequentially disposed fishponds with continuous water flow. Braz J Biol 67:643–649

Smith S, Renwick W, Bartley J, Buddemeier R (2002) Distribution and significance of small, artificial water bodies across the United States landscape. Sci Total Environ 299:21–36. https://doi.org/10.1016/s0048-9697(02)00222-x

Soares FS, Almeida RK, Rubim IB et al (2015) Análise comparativa da correção atmosférica de imagem do Landsat 8: o uso do 6S e do ATCOR2. In: Gherardi, D. F. M., Aragão, L. E. O. C. (ed). Simpósio Brasileiro de Sensoriamento Remoto. *INPE*, João Pessoa, 1821–1825

Sobral FL, Cianciaruso MV (2012) Estrutura filogenética e funcional de assembleias: (re)montando a Ecologia de Comunidades em diferentes escalas espaciais. Bioscience Journal 28:4. https://seer.ufu.br/index.php/biosciencejournal/article/view/13491

SOS Mata Atlântica, INPE - Instituto Nacional de Pesquisas Espaciais (2008) Atlas dos remanescentes florestais da Mata Atlântica, período de 2000 a 2005. https://www.sosmatatlantica.org.br. Acessed on 20 February 2020.

Sun Z, Zhao C, Zhu W et al (2021) Microhabitat features determine the tadpole diversity in mountainous streams, Ecological Indicators 126:107647. https://doi.org/10.1016/j.ecolind.2021.107647

Ternus RZ, Souza-Franco GM, Anselmini MEK et al (2011) Influence of urbanization on water quality in the basin of the upper Uruguay River in western Santa Catarina, Brazil. Acta Limnol Bras 23:189–199

Thomaz SM, Cunha ER (2010) The role of macrophytes in habitat structuring in aquatic ecosystems: methods of measurement, causes and consequences on animal assemblages composition and biodiversity. Acta Limnol Bras 22:218–236. https://doi.org/10.4322/actalb.02202011

Tilman D, Knops J, Wedin D et al (1997) The influence of functional diversity and composition on ecosystem processes. Science 277:1300–1302

Vasconcelos TDS, Rossa-Feres DDC (2005) Diversidade, distribuição espacial e temporal de anfíbios anuros (Amphibia, Anura) na região noroeste do estado de São Paulo, Brasil. Biota Neotrop 5:137–150

Veloso HP, Goés-Filho L (1982) Fitogeografia brasileira: classificação fisionômico-ecológica da vegetação neotropical. Ministério das Minas e Energia-Secretaria Geral-Projeto RADAM BRASIL, Salvador

Violle C, Navas ML, Vile D et al (2007) Let the concept of trait be functional! Oikos, 116:882-892. https://doi.org/10.1111/j.2007. 0030-1299.15559.x

Violle C, Enquist BJ, McGill BJ et al (2012) The return of the variance: intraspecific variability in community ecology. Trends Ecol Evol 27:244-252. https://doi.org/10.1016/j.tree.2011.11.014

Weiher E, Freund D, Bunton T et al (2011) Advances, challenges and a developing synthesis of ecological community assembly theory. Philos Trans R Soc Lond B Biol Sci 366:2403–2413. https://doi.org/10.1098/rstb.2011.0056

Wellborn GA, Skelly DK, Werner EE (1996) Mechanisms creating community structure across a freshwater habitat gradient. Annu Rev Ecol Evol Syst 27:337–363

Werner EE, Yurewicz KL, Skelly DK et al (2007) Turnover in an amphibian metacommunity: the role of local and regional factors. Oikos 116:1713–1725. https://doi.org/10.1111/j.0030-1299.2007.16039.x

Williams BK, Rittenhouse TAG, Semlitsch RD (2008) Leaf litter input mediates tadpole performance across forest canopy treatments. Oecologia 155:377–384. https://doi.org/10.1007/s00442-007-0920-y

Zongo B, Boussim JI (2015) The effects of physicochemical variables and tadpole assemblages on microalgal communities in freshwater temporary ponds through an experimental approach. Aquat Biosyst 11:1. https://doi.org/10.1186/s12999-014-0013-4

Supplementary Information

Trait-environment relationship in tadpole communities of the southern Atlantic Forest

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Table S1 Tadpole abundance for 28 waterbodies in areas of Atlantic Forest in southern Brazil, recorded from October 2018 to March 2019. P = Pond; S = Stream.

Family/Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	P	S	S	P	S	P	S	P	S	P	S	P	S	P
BUFONIDAE														
Rhinella henseli (Lutz, 1934)	0	0	0	0	119	0	0	0	0	0	12	0	0	0
Rhinella icterica (Spix, 1824)	0	0	0	0	0	400	1	0	0	0	0	40	0	0
HYLIDAE														
Aplastodiscus perviridis Lutz 1950	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Boana cf. *curupi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Boana curupi (Garcia, Faivovichi and Haddad, 2007)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Boana faber (Wied - Neuwied 1821)	0	0	0	0	0	0	0	11	1	53	1	0	0	124
Boana leptolineata (Braun and Braun, 1977)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Boana prasina (Burmeister, 1856)	0	67	16	0	0	0	0	0	0	0	0	0	0	0
Boana pulchella (Duméril and Bibron,1841)	0	9	4	0	0	0	0	0	0	0	0	0	0	0
Dendropsophus microps (Peters, 1872)	0	0	0	0	12	0	0	0	0	1	4	0	9	0
Dendropsophus minutus (Peters, 1872)	5	0	0	0	0	0	0	21	0	0	0	5	14	18
Scinax fuscovarius (Lutz, 1925)	0	0	0	3	0	0	0	28	0	0	0	0	9	0
Scinax granulatus (Peters, 1871)	0	0	3	0	0	0	0	3	0	0	0	5	9	0
Scinax perereca Pombal, Haddad and Kasahara, 1995	0	0	0	8	0	0	0	1	0	0	2	0	0	0

HYLODIDAE														
Crossodactylus schmidti Gallardo, 1961	0	0	0	0	0	0	124	0	55	0	0	0	0	0
LEPTODACTYLIDAE														
Leptodactylus latrans (Steffen, 1815)	0	0	0	0	0	0	0	0	0	19	0	0	0	0
Physalaemus cuvieri Fitzinger, 1826	6	0	0	1	39	0	0	0	0	6	0	110	53	0
Physalaemus cf. *carrizorum*	0	0	0	0	0	0	0	0	0	0	0	2	11	0
MICROHYLIDAE														
Elachistocleis bicolor (Guérin-Méneville, 1838)	0	0	0	0	0	0	0	2	0	0	0	0	0	0
ODONTOPHRYNIDAE														
Proceratophrys avelinoi Mercadal de Barrio and Barrio, 1993	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHYLLOMEDUSIDAE														
Phyllomedusa tetraploidea Pombal and Haddad, 1992	0	0	0	88	0	0	0	7	0	0	0	0	0	0
RANIDAE														
Lithobates catesbeianus (Shaw 1802)	0	0	0	0	0	0	0	25	0	10	0	0	0	0
Total abundance	11	76	23	100	170	400	126	98	56	89	19	162	105	142
Total richness	2	2	3	3	3	1	3	8	2	5	4	5	6	2
Total														

Continuation

Table S1 Tadpole abundance for 28 waterbodies in areas of Atlantic Forest in southern Brazil, recorded from October 2018 to March 2019. P = Pond; S = Stream.

Family/Species	15	16	17	18	19	20	21	22	23	24	25	26	27	28
	S	S	P	S	P	S	S	P	S	S	P	S	P	S
BUFONIDAE														
Rhinella henseli (Lutz, 1934)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhinella icterica (Spix, 1824)	0	0	0	19	0	0	0	0	0	0	0	0	0	0
HYLIDAE														
Aplastodiscus perviridis Lutz 1950	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Boana cf. *curupi*	0	0	0	0	0	0	55	0	30	37	0	0	0	0
Boana curupi (Garcia, Faivovichi and Haddad, 2007)	50	98	0	0	0	0	43	0	6	41	0	0	0	0
Boana faber (Wied - Neuwied 1821)	1	0	14	91	48	0	0	10	0	0	40	6	0	0
Boana leptolineata (Braun and Braun, 1977)	3	6	0	0	0	0	0	0	0	0	0	0	0	0
Boana prasina (Burmeister, 1856)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Boana pulchella (Duméril and Bibron,1841)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dendropsophus microps (Peters, 1872)	0	0	0	0	7	0	0	40	0	0	14	0	0	0
Dendropsophus minutus (Peters, 1872)	1	0	0	0	53	2	0	0	0	0	2	0	0	0
Scinax fuscovarius (Lutz, 1925)	0	0	0	0	9	0	0	5	0	0	0	0	0	0
Scinax granulatus (Peters, 1871)	2	0	0	0	0	0	0	0	0	0	0	0	0	0

Scinax perereca Pombal, Haddad and Kasahara, 1995	0	0	0	0	0	0	0	9	0	0	0	0	90	67
HYLODIDAE														
Crossodactylus schmidti Gallardo, 1961	0	0	0	0	0	0	16	0	0	0	0	118	0	0
LEPTODACTYLIDAE														
Leptodactylus latrans (Steffen, 1815)	0	0	0	53	0	0	0	0	0	0	0	0	0	0
Physalaemus cuvieri Fitzinger, 1826	0	0	0	0	28	64	0	0	0	0	0	0	0	0
Physalaemus cf. *carrizorum*	0	0	0	0	0	0	0	8	0	0	0	0	0	0
MICROHYLIDAE														
Elachistocleis bicolor (Guérin-Méneville, 1838)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ODONTOPHRYNIDAE														
Proceratophrys avelinoi Mercadal de Barrio and Barrio, 1993	0	0	7	0	0	0	0	0	0	0	0	0	0	0
PHYLLOMEDUSIDAE														
Phyllomedusa tetraploidea Pombal and Haddad, 1992	6	0	0	0	0	0	0	19	0	0	56	0	0	0
RANIDAE														
Lithobates catesbeianus (Shaw 1802)	0	0	0	0	2	0	0	8	0	0	0	0	0	0
Total abundance	63	104	21	163	147	66	114	99	36	78	112	124	90	67
Total richness	6	2	2	2	6	2	3	7	2	2	4	2	1	1
Total														2,861

Table S2 Summary of Monte-Carlo tests of RLQ models of ponds.

Model	Functional traits selected	Predictors selected	Standard observed	P-value
Local environmental descriptors – global model	All traits	All predictors	3.021	0.02
Local environmental descriptors – fitted model	BH; BL; BW; TMW; DFH; VFH; NTR; ODP; MS; ES; SL; SW; ND; IED; Fl; EP; SP; NP	Depth; water pH; water temperature; water conductivity; pond vegetation	3.687	0.001
Landscape descriptors – global model	All traits	All predictors	1.523	0.06
Landscape descriptors – fitted model	BH; BL; BW; TMW; DFH; VFH; NTR; ODP; MS; ES; SL; SW; ND; IED; Fl; EP; SP; NP	Forest; agriculture; pasture; urban; edge dist.; types of land use	1.051	0.15

Table S3 Relationships between pond variables and RLQ axes in the fitted model. Q1 = Axis 1; Q2= Axis 2; Local environment descriptor = Local physicochemical and morphological characteristics; Std. obs. values = Standard observed values; Adj. p-value = Adjusted p-value.

RLQ axes	Local environment descriptor	Std. obs. values	Adj. p-value
Q1	Water depth	-2.570	0.006
	Water pH	-2.346	0.008
	Water temperature	-1.333	0.20
	Water conductivity	1.287	0.20
	Pond grass	-0.462	0.32
	Pond grass+monocultures	2.384	0.94
	Pond grass+trees	0.401	0.62
	Pond shrubs	-0.535	0.38
	Pond trees	-0.916	0.17
Q2	Water depth	-0.270	0.80
	Water pH	0.576	0.58
	Water temperature	1.336	0.58
	Water conductivity	-1.650	0.05
	Pond grass	0.191	0.60
	Pond grass+monocultures	-0.408	0.5
	Pond grass+trees	0.661	0.75
	Pond shrubs	-0.719	0.29
	Pond trees	-1.397	0.05

Table S4 Relationship between the tadpole functional traits and RLQ axes in the fitted model of ponds (model with local environmental descriptors); R1 = Axis 1; R2 = Axis 2; Functional traits = Measured characteristics of tadpoles; Std. obs. values = Standard observed values; Adj. p-value = Adjusted p-value.

RLQ axes	Functional traits	Std. obs. values	Adj. p-value
R1	BH	-0.382	0.72
	BL	1.649	0.09
	BW	2.027	0.05
	TMW	-1.752	0.05
	DFH	2.121	0.02
	VFH	0.856	0.44
	NTR	-0.309	0.75
	ODP anterior	-0.302	0.41
	ODP anteroventral	-0.031	0.47
	ODP ventral	0.00	0.99
	MS	-1.777	0.05
	ES	-1.883	0.05
	SL	0.561	0.62
	SW	0.903	0.39
	ND	-0.991	0.33
	IED	-2.145	0.02
	Fl absent	-1.985	0.04
	Fl present	0.759	0.77
	EP dorsal	-2.148	0.009
	EP dorsolateral	0.110	0.56
	EP lateral	-0.003	0.43
	SP posterior	-0.528	0.35
	SP posterodorsal	-2.417	0.02
	SP sinistral	0.000	0.99
	SP ventral	0.547	0.75
	NP anteroventral	-0.496	0.38
	NP absent	0.016	0.57
	NP dorsal	-0.601	0.33

	NP dorsolateral	-1.145	0.12
	NP lateral	-1.010	0.16
	NP laterodorsal	0.000	0.99
R2	BH	1.846	0.05
	BL	0.110	0.92
	BW	-1.264	0.23
	TMW	1.129	0.26
	DFH	1.403	0.17
	VFH	0.112	0.91
	NTR	-0.913	0.40
	ODP anterior	0.369	0.65
	ODP anteroventral	-0.763	0.24
	ODP ventral	0.000	1.00
	MS	1.458	0.15
	ES	0.695	0.49
	SL	-0.315	0.79
	SW	0.946	0.36
	ND	1.220	0.23
	IED	0.540	0.59
	Fl absent	-1.844	0.04
	Fl present	2.127	0.98
	EP dorsal	-0.806	0.24
	EP dorsolateral	-0.325	0.38
	EP lateral	0.734	0.75
	SP posterior	0.178	0.60
	SP posterodorsal	-2.556	0.01
	SP sinistral	0.000	1.00
	SP ventral	1.709	0.95
	NP anteroventral	-0.017	0.61
	NP absent	0.572	0.69
	NP dorsal	-1.163	0.11
	NP dorsolateral	-1.607	0.05
	NP lateral	-0.795	0.22

	NP laterodorsal	0.000	1.00

Table S5 Pond landscape descriptors and RLQ axes; Q1 = Axis 1; Q2 = Axis 2; Landscape descriptor = Landscape descriptor data; Std. obs. values = Standard observed values; Adj. p-value = Adjusted p-value.

RLQ axes	Landscape descriptor	Std. obs. values	Adj. p-value
Q1	Forest	-0.205	0.84
	Agriculture	0.687	0.52
	Pasture	-0.119	0.93
	Urban	-2.780	0.001
	Edge dist.	-2.621	0.003
	Canopy open	-1.073	0.12
Q2	Forest	1.988	0.03
	Agriculture	-2.594	0.01
	Pasture	0.553	0.60
	Urban	0.647	0.48
	Edge dist.	0.230	0.87
	Canopy open	-1.843	0.10

Table S6 Relationship between the tadpole functional traits and RLQ axes in the fitted model of ponds (model with landscape descriptors); R1 = Axis 1; R2= Axis 2; Functional traits = Measured characteristics of tadpoles; Std. obs. values = Standard observed values; Adj. p-value = Adjusted p-value.

RLQ axes	Functional traits	Std. obs. values	Adj. p-value
R1	BH	1.397	0.16
	BL	-0.493	0.68
	BW	-1.565	0.14
	TMW	1.069	0.32
	DFH	0.159	0.89
	VFH	0.484	0.62
	NTR	-0.643	0.64
	ODP anterior	-0.324	0.45
	ODP anteroventral	-0.827	0.28

	ODP ventral	<0.001	1.00
	MS	1.397	0.16
	ES	1.526	0.10
	SL	0.390	0.58
	SW	1.088	0.26
	ND	1.295	0.23
	IED	1.437	0.12
	Fl absent	0.767	0.70
	Fl present	-0.729	0.34
	EP dorsal	0.282	0.64
	EP dorsolateral	1.252	0.89
	EP lateral	1.408	0.14
	SP posterior	0.014	0.63
	SP posterodorsal	0.086	0.45
	SP sinistral	<0.001	1.00
	SP ventral	-0.653	0.30
	NP anteroventral	-0.469	0.48
	NP absent	-0.598	0.27
	NP dorsal	0.386	0.62
	NP dorsolateral	-0.166	0.61
	NP lateral	-0.238	0.51
	NP laterodorsal	<0.001	1.00
R2	BH	0.239	0.84
	BL	0.174	0.86
	BW	0.148	0.88
	TMW	0.236	0.84
	DFH	2.319	0.02
	VFH	0.681	0.52
	NTR	-0.051	0.95
	ODP anterior	-1.042	0.16
	ODP anteroventral	1.539	0.93
	ODP ventral	<0.001	1.00
	MS	0.285	0.79

ES	-0.071	0.94
SL	0.877	0.39
SW	1.223	0.23
ND	0.874	0.40
IED	-1.148	0.25
Fl absent	-2.347	0.02
Fl present	1.603	0.94
EP dorsal	-1.588	0.04
EP dorsolateral	-0.688	0.23
EP lateral	1.902	0.98
SP posterior	-1.370	0.11
SP posterodorsal	-1.773	0.05
SP sinistral	<0.001	1.00
SP ventral	0.591	0.74
NP anteroventral	-0.662	0.38
NP absent	-0.171	0.47
NP dorsal	-1.593	0.05
NP dorsolateral	-0.135	0.36
NP lateral	0.113	0.51
NP laterodorsal	<0.001	1.00

Table S7 Summary of Monte-Carlo tests of RLQ models of streams.

Model	Functional traits selected	Predictors selected	Standard observed	P-value
Local environmental descriptors – global model	All traits	All predictors	3.008	0.003
Local environmental descriptors – fitted model	BH; BL; BW; TMW; DFH; VFH; NTR; ODP; MS; ES; SL; SW; ND;	Area; water transp.; water pH; water temperature; water conductivity; diss. ox.; DQO; NO_2; Total alk.;	3.543	0.001

	IED; Fl; EP; SP; NP	alk HCO_3; stream vegetation; stream substrate		
Landscape descriptors – global model	All traits	All predictors	1.208	0.11
Landscape descriptors – fitted model	BH; BL; BW; TMW; DFH; VFH; NTR; ODP; MS; ES; SL; SW; ND; IED; Fl; EP; SP; NP	Canopy open; types of land use	0.341	0.35

Table S8 Relationship between the stream local descriptors and RLQ axes of the fitted model. Q1 = Axis 1; Q2 = Axis 2; Local environment descriptor = Local physicochemical and morphological characteristics; Std. obs. values = Standard observed values; Adj. p-value = Adjusted p-value.

RLQ axes	Local environment descriptor	Std. obs. values	Adj. p-value
Q1	Stream area	-0.916	0.38
	Water transparency	-0.904	0.39
	Water pH	-2.453	0.008
	Water temperature	-2.397	0.01
	Dissolved oxygen	-1.013	0.33
	Water conductivity	2.162	0.01
	COD	0.191	0.87
	NO_2	1.358	0.18
	Total alk	2.122	0.03
	Alk HCO_3	2.122	0.03
	Stream grass	0.188	0.81
	Stream grass+trees	1.685	0.88
	Stream shrubs	-0.818	0.29
	Stream trees	-0.973	0.14

	Stream aquatic veg.	0.933	0.78
	Stream grass+rocks	-0.811	0.16
	Stream leaves+roots+rocks	-0.747	0.31
	Stream mud	1.173	0.80
	Stream mud+rocks	-0.854	0.04
	Stream rocks	0.000	1.00
	Stream roots+roocks	-0.834	0.20
Q2	Stream area	-1.542	0.12
	Water transparency	-0.781	0.46
	Water pH	1.301	0.20
	Water temperature	1.314	0.20
	Dissolved oxygen	0.496	0.65
	Water conductivity	1.471	0.15
	COD	-0.105	0.91
	NO_2	1.110	0.28
	Total alk	0.612	0.58
	Alk HCO_3	0.612	0.58
	Stream grass	-0.864	0.30
	Stream grass+trees	1.036	0.90
	Stream shrubs	1.403	0.88
	Stream trees	-2.112	0.03
	Stream aquatic veg.	0.989	0.85
	Stream grass+rocks	-1.087	0.04
	Stream leaves+roots+rocks	-1.078	0.19
	Stream mud	-0.783	0.32
	Stream mud+rocks	0.354	0.69
	Stream rocks	0.000	1.00
	Stream roots+roocks	-0.980	0.14

Table S9 The relationship between tadpole functional traits and RLQ axes of the fitted model which included tadpoles in streams; R1 = Axes 1; R2 = Axes 2; Std. obs. values = Standard observed values; Adj. p-value = Adjusted p-value.

RLQ axes	Environment descriptor	Std. obs. values	Adj. p-value

R1	BH	0.591	0.56
	BL	-1.873	0.05
	BW	-2.127	0.02
	TMW	2.136	0.02
	DFH	-0.645	0.55
	VFH	1.015	0.32
	NTR	0.005	0.99
	ODP anterior	-0.768	0.03
	ODP anteroventral	0.049	0.36
	MS	1.537	0.11
	ES	1.868	0.05
	SL	-1.104	0.28
	SW	-0.226	0.81
	ND	1.754	0.07
	IED	1.751	0.07
	Fl absent	-1.292	0.14
	Fl present	0.766	0.74
	EP dorsal	-1.417	0.08
	EP dorsolateral	1.100	0.87
	EP lateral	0.789	0.75
	SP posterior	0.222	0.67
	SP posterodorsal	-0.551	0.24
	SP ventral	0.738	0.79
	NP anteroventral	0.738	0.79
	NP dorsal	-1.849	0.03
	NP dorsolateral	-0.492	0.35
	NP lateral	0.060	0.60
	BH	-0.521	0.64
	BL	-0.591	0.57
	BW	0.236	0.82
	TMW	-1.062	0.30
	DFH	1.449	0.14
	VFH	1.572	0.12

R2	NTR	-1.422	0.16
	ODP anterior	-0.785	0.23
	ODP anteroventral	-1.370	0.11
	MS	-0.911	0.38
	ES	-0.916	0.37
	SL	-1.580	0.12
	SW	0.060	0.96
	ND	-1.342	0.19
	IED	-1.342	0.18
	Fl absent	-0.648	0.26
	Fl present	0.982	0.84
	EP dorsal	-1.594	0.04
	EP dorsolateral	1.440	0.93
	EP lateral	0.811	0.79
	SP posterior	0.182	0.60
	SP posterodorsal	-0.597	0.26
	SP ventral	0.968	0.82
	NP anteroventral	0.986	0.82
	NP dorsal	-1.925	0.02
	NP dorsolateral	1.137	0.87
	NP lateral	-0.189	0.41

Table S10 Relationship between the stream landscape descriptors and RLQ axes of the fitted model. Q1 = Axes 1; Q2 = Axes 2; Environment Descriptor = environment descriptor; Std. obs. values = Standard observed values; Adj. p-value = Adjusted p-value.

RLQ axes	Environment descriptor	Std. obs. values	Adj. p-value
Q1	Forest	-1.491	0.14
	Agriculture	-1.427	0.15
	Pasture	0.368	0.72
	Urban	2.063	0.03
	Edge dist.	1.796	0.08
	Canopy open	1.758	0.08
Q2	Forest	-0.838	0.42

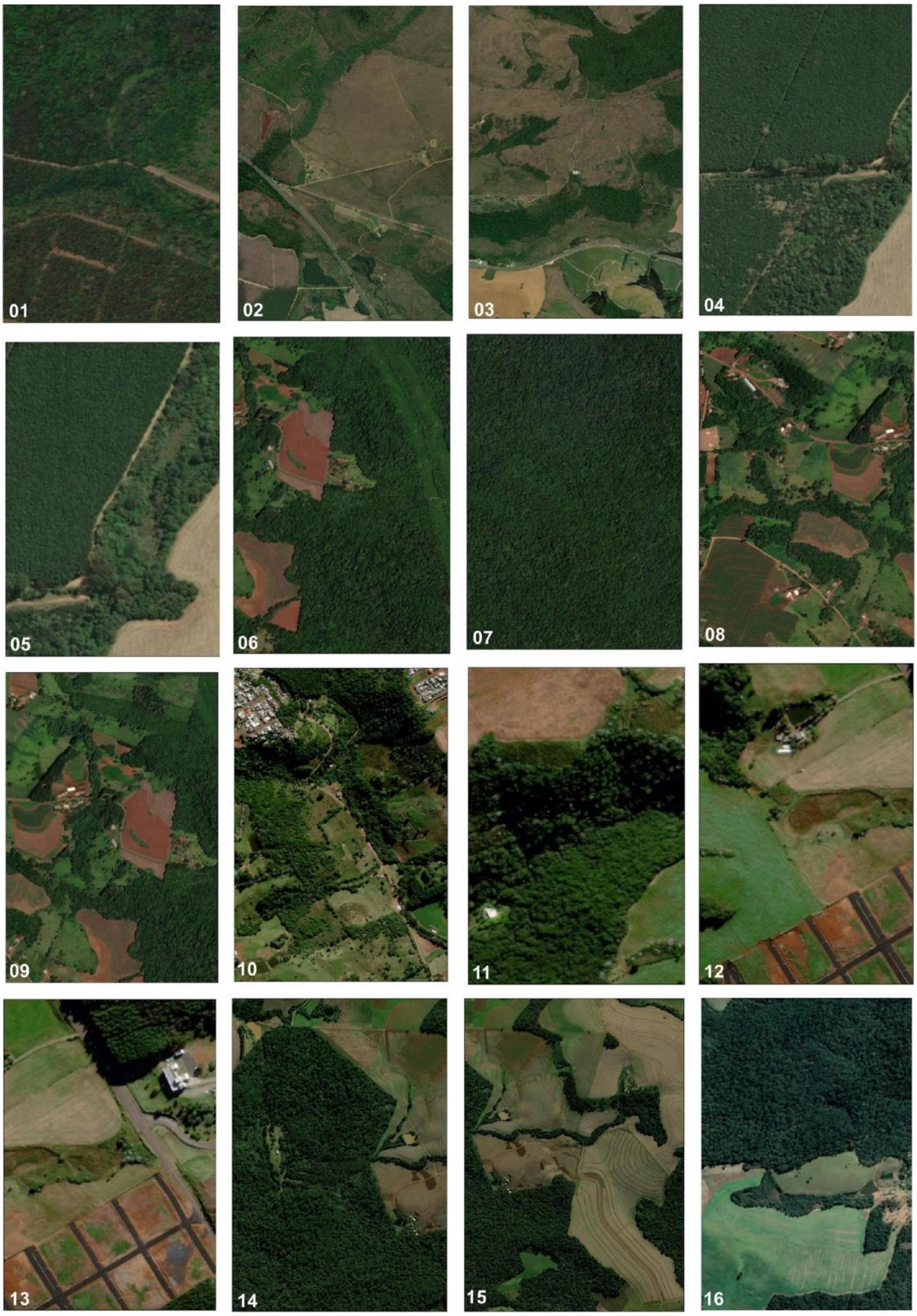
	Agriculture	0.808	0.43
	Pasture	-1.646	0.10
	Urban	-1.238	0.18
	Edge dist.	-1.444	0.16
	Canopy open	1.438	0.15

Table S11 Relationship between the tadpole functional traits and RLQ axes in the fitted model of streams (model with landscape descriptors); R1 = Axes 1; R2 = Axes 2; Environment Descriptor = environment descriptor; Std. obs. values = Standard observed values; Adj. p-value = Adjusted p-value.

RLQ axes	Environment descriptor	Std. obs. values	Adj. p-value
R1	BH	1.067	0.31
	BL	-2.236	0.02
	BW	-1.909	0.05
	TMW	1.976	0.04
	DFH	-0.769	0.46
	VFH	1.536	0.12
	NTR	-0.896	0.38
	ODP anterior	-0.818	0.09
	ODP anteroventral	-1.205	0.13
	MS	1.066	0.31
	ES	1.792	0.07
	SL	-1.989	0.03
	SW	-0.646	0.55
	ND	1.815	0.05
	IED	1.689	0.08
	Fl absent	-1.100	0.14
	Fl present	-0.514	0.27
	EP dorsal	-1.553	0.08
	EP dorsolateral	0.784	0.78
	EP lateral	-0.494	0.27
	SP posterior	1.917	0.95
	SP posterodorsal	-1.756	0.04

	SP ventral	-0.358	0.42
	NP anteroventral	-0.358	0.42
	NP dorsal	-1.928	0.03
	NP dorsolateral	-0.648	0.28
	NP lateral	-0.215	0.40
R2	BH	0.057	0.96
	BL	0.031	0.98
	BW	-0.199	0.85
	TMW	1.729	0.07
	DFH	-0.823	0.41
	VFH	-0.861	0.40
	NTR	2.187	0.02
	ODP anterior	-0.737	0.12
	ODP anteroventral	-0.791	0.18
	MS	1.834	0.05
	ES	1.390	0.18
	SL	0.922	0.38
	SW	-0.516	0.64
	ND	1.612	0.11
	IED	1.619	0.10
	Fl absent	-1.435	0.09
	Fl present	1.626	0.92
	EP dorsal	-1.608	0.02
	EP dorsolateral	1.488	0.93
	EP lateral	1.627	0.93
	SP posterior	-0.542	0.39
	SP posterodorsal	-0.725	0.24
	SP ventral	2.099	0.95
	NP anteroventral	2.099	0.95
	NP dorsal	-1.689	0.03
	NP dorsolateral	0.137	0.62
	NP lateral	-1.274	0.05

Figure S1 Photographic images showing details of the landscape of each waterbody sampled in the tadpole collection in the areas of Atlantic Forest, in southern Brazil.

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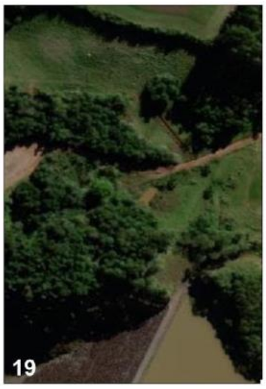
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CAPÍTULO 4 - Effects of landscape on the incidence of infectious diseases (*Bd* and ranavirus) in tadpoles in the southern Atlantic Forest

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Highlights

- Chytridiomycosis (*Bd*) is widespread in tadpoles in the southern Atlantic Forest
- Communities had more *Bd* infections than expected in the southern Atlantic Forest
- *Bd* infections are associated with larger forest areas but not with land use
- The anurans *Boana curupi*, *B. faber* and *Crossodactylus schmidti* are *Bd* indicators
- Ranavirus is not currently, but potentially a threat to the evaluated communities

Abstract

Together with habitat loss, infectious diseases have been one of the main reasons for the worldwide decline of amphibian populations. The search for biotic indicators is a useful tool for mapping priority demands in disease management. Here, we used indicators at both species and community levels to investigate the incidence of two pathogens, the *Batrachochytrium dendrobatidis* (*Bd*) and ranavirus, in anuran communities in southern Brazil. Our data, based on 26 anuran communities, revealed that ranavirus deserves less attention than *Bd* in terms of management efforts. On the other hand, the prevalence and load of *Bd* were higher than that observed in other Atlantic Forest communities. Three species were pointed as chytrid indicators: *Boana curupi*, *Crossodactylus schmidti* (threatened and endemic to southern Brazil and Argentina) and *Boana faber*. Additionally, the analysis at the community level revealed that communities from larger forest areas are *Bd* indicators. This is novel and very relevant information since most of the Atlantic Forest consists of a fragmented landscape (patched habitat). Based on our results, we argue that anurans from larger (apparently well preserved) forests are more prone to be affected by *Bd* and that the use of *Bd*-indicator species and *Bd*-indicator communities is a useful approach for conservation and landscape-management proposals.

Keywords: Amphibian, chytridiomycosis, diseases, indicator species, remnants forest, tadpoles

Introduction

Environmental changes in habitat can increase the susceptibility of wild species to develop emerging infectious diseases (Preuss et al., 2020; Pereira da Silva et al., 2022). This is a secondary indirect but powerful effect of human activities on biodiversity (Fisher and Garner, 2020). The impact of these diseases on native species depends on the speed at which pathogens are spread, combined with the host's susceptibility to infections (Fisher and Garner, 2020). Host susceptibility can increase due to changes in the pathogen-host dynamics promoted by habitat changes (e.g., deforestation, pollution, microclimatic changes; Gillespie et al., 2005; Kärvemo et al., 2019; McMillan et al., 2020) which makes an evaluation at species, communities, and landscape levels important. Changes in the landscape can increase the emergence of new pathogens or the re-emergence of more virulent forms of pre-existing pathogens (Jones et al., 2008; Giam, 2017; Pereira da Silva et al., 2022). This is of high concern in tropical regions, which harbour high biodiversity (Mittermeier et al., 2005, 2011), and where a continuous landscape change is noted, in particular driven by economic development in some countries.

Emerging infectious diseases have been reported as important threats to amphibians (Latney and Klaphake, 2020), and chytridiomycosis is of particular concern due to its high lethality. Chytridiomycosis, caused by the fungi *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*) (Longcore et al., 1999; Martel et al., 2013), has caused population declines in more than 500 amphibian species in the world (Scheele et al., 2019; Fisher and Garner, 2020), making it a worldwide threat for amphibians (O'Hanlon et al., 2018). *Bd* parasitizes any amphibian life stage (Rachowicz and Vredenburg, 2004) and causes epidermal hyperplasia, hyperkeratosis and/or death in adults (Pessier et al., 1999; Nichols et al., 2001), dekeratinization of the corneous beak in tadpoles, which can result in deformities in the oral apparatus (Knapp and Morgan, 2006), and sublethal effects in tadpoles (Hanlon et al., 2015).

The impact of *Bd* in a particular population varies according to the anuran biology, reproductive behaviour and skin microbiome (Ruggeri et al., 2018; Sapsford et al., 2018). However, infection levels also depend on the community configuration (e.g., species composition) and environmental factors (see Becker et al., 2015). As environmental factors, we can mention vegetation cover (Hernández-López et al., 2018; García-Feria et al., 2019), canopy cover (Becker and Zamudio, 2011; Delazeri et al., 2021), habitat integrity (Becker et al., 2009), configuration of waterbodies (Ruggeri et al., 2018; Kärvemo et al., 2019), water characteristics (Piotrowski et al., 2004; Simpkins et al., 2017; Preuss et al., 2020), temperature (Piotrowski et al., 2004; Sonn et al., 2017), topography and latitude (Piovia-Scott et al., 2011; Lambertini et al., 2021).

Studies showed that high levels of *Bd* infection are observed even in well-preserved habitats so even organisms in undisturbed habitats are susceptible (Valencia-Aguilar et al., 2016; Delazeri et al., 2021; Lambertini et al., 2021). Some cues about habitat proprieties that facilitate *Bd* infection have been shared by researchers. Among them, shading and low temperatures seem to favour the development of the fungus (Valencia-Aguilar et al., 2016; Delazeri et al., 2021). This makes us believe that subtropical forests, such as those in southern Brazil, are potential hotspots for *Bd* in Neotropics, as they fit the expected adequacy for the fungus (Jenkinson et al., 2016). In fact, a high prevalence of the fungus has been recorded in the southern (subtropical) domain of the Atlantic Forest (Ernetti et al., 2020; Delazeri et al., 2021).

In Brazil, *Bd* records in the Atlantic Forest are relatively well mapped (Preuss et al., 2016; Ernetti et al., 2020; Santos et al., 2020; Delazeri et al., 2021; Lambertini et al., 2021). On the other hand, few data have been generated regarding Ranavirus, which is another important infection (Ruggeri et al., 2019). Ranavirus, which affects not exclusively amphibians but also fishes and reptiles, deserves attention since it spreads quickly, causes high lethality, and has already led populations to extinction (Daszak et al., 1999; Green et al., 2002), so it is another important threat to amphibians (Miller et al., 2011; Ruggeri et al., 2019). Despite this, some ranavirus lineages, such as the "FV3 (*Ranavirus: Iridoviridae*), are widely distributed and have already been detected in about 200 species (Duffus et al., 2015; Chinchar et al., 2017). Current evidence suggests that the susceptibility to this disease varies among groups that differ in terms of phylogenetic lineages (Schock et al., 2010; Hoverman et al., 2011), but it has the potential to infect species worldwide (see Gray and Chinchar, 2015).

As the Atlantic Forest encompasses high species diversity of anurans and high endemism (Mittermeier et al., 2011; Haddad et al., 2013), this is a biome of great concern regarding extinctions risks. Mortality by *Bd* is relatively rare in tadpoles (Garner et al., 2009; Gervasi et al., 2013; Martel et al., 2013), but massive mortality by ranavirus has been reported (Rijks et al., 2016; Ruggeri et al., 2019). *Bd* and ranavirus seem to vary in terms of mortality rate among tadpoles and adults. Ranavirus can be a threat to the survival of tadpoles, while *Bd* represents a similar threat to adults. However, tadpoles could act as a reservoir and vector of *Bd* (Daszak et al., 2003; Rachowicz and Vredenburg, 2004; Blaustein et al., 2005; Narayan et al., 2014; Valencia-Aguilar et al., 2016). Thus, the association of ranavirus and *Bd* can be highly problematic for the maintenance of amphibian populations due to their combined/complementary impact over all stages of their life. Due to the great variety of responses at species, organism, and community levels, we propose herein a search for indicators of biotic suitability to *Bd* and ranavirus infections. To achieve this, we analysed the incidence of *Bd* and ranavirus in different tadpole communities in the subtropical domain of the Atlantic Forest of southern Brazil. We explored the relationship between species composition, local habitat, and landscape configuration over the prevalence, loads, and frequency of these diseases on individuals based on a community-indicator and species-indicator approach.

Material and methods

Study Area

We conducted this study in the subtropical Atlantic Forest, southern Brazil, between coordinates 22º30' N to 33º45' S and 48º02' E to 57º40' W, which encompasses three Brazilian states: Paraná, Santa Catarina and Rio Grande do Sul (Fig. 1). This region was originally covered by different phytophysiognomies of the Atlantic Forest: Mixed Ombrophilous Forest (Araucaria Forest), Seasonal Forest, and High-Altitude Grasslands (Veloso and Góes-Filho, 1982; https://www.sosmatatlantica.org.br). Currently, remnants of these phytophysiognomies share space whit urban, livestock-raising, and agricultural areas (Ribeiro et al., 2009; Pillar and Vélez, 2010).

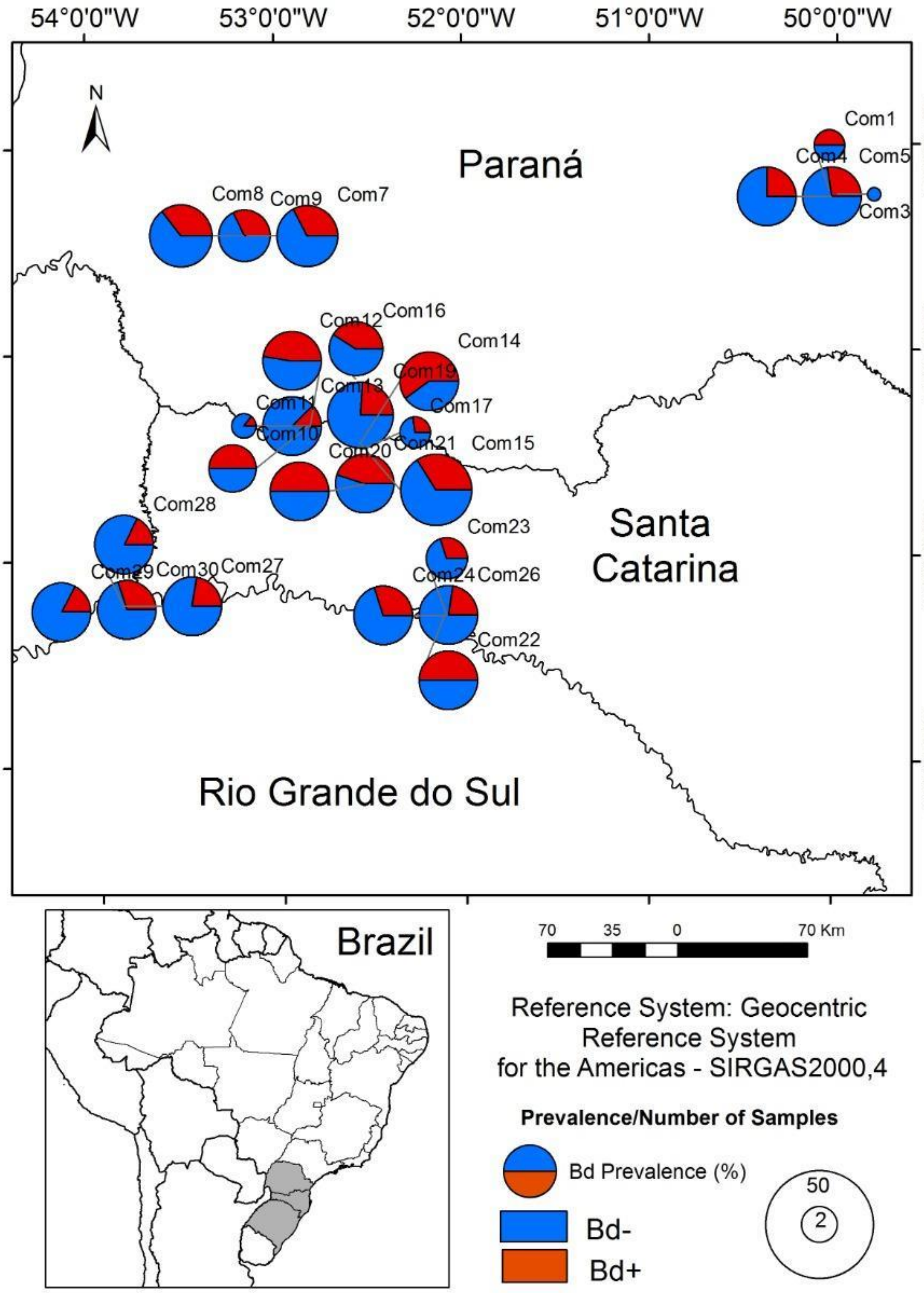

Fig. 1. Location of the sampling areas and *Bd* prevalence in the tadpole communities in southern Brazil (More details in Table S1). Colours show the proportion of infected (*Bd*+) and non-infected (*Bd*-) tadpoles.

The climate is subtropical, with annual air temperatures varying from 16 °C to 24

°C and rainfall varies from 1,600–2,200 mm annually (Alvares et al., 2013). We selected sampling areas based on: (1) presence of well-preserved native forest; (2) similar climatic conditions; (3) elevation between 300 m and 900 m above sea level; (4) similar topography; and (5) presence of lentic and lotic waterbodies used as breeding sites by anurans. After a search based on satellite images followed by "*in loco*" inspections, we selected seven forest areas in which we defined waterbodies as sampling points. We sampled at least one lentic and one lotic waterbody in each of the seven areas to assess a wide variety of habitats and tadpole communities (Melo et al., 2017). We sampled a total of 26 waterbodies, of which 12 are ponds (permanent or temporary) and 14 streams associated with the forest, with stretches of up to 100 meters in length (Table S1). The streams were of low order, with a width of 1.99 ± 0.79 (standard deviation, SD) m (range, 0.60–3.50 m), and the polygon area of the ponds was 386.45 ± 307.39 m². Streams had a mean depth of 0.45 ± 0.12 m (range 0.15–0.70 m) and ponds had a mean depth of 0.8 ± 0.40 m (range 0.30–1.50). The vegetation surrounding the waterbodies consisted of grasses, shrubs, and trees, having distinct compositions (see Supplementary Materials, Table S1).

Tadpole collection

We sampled all waterbodies from October 2018 to March 2019. This period includes the peak of the breeding season of most species. The study site is under a climatic regime with a relatively cold and harsh winter for tropical anurans, which restricts their reproduction to hotter and more humid months (Preuss et al., 2020; Santos et al., 2021). The whole sampled area is distributed across about 10,500,000 ha. This wide extension made repeated sampling difficult, and we sampled each waterbody only once. We performed tadpole sampling from 0800–1800 using a 3-mm²-mesh dip-net (Heyer 1976).

Due to the large distance between the waterbodies, each one was considered a source of a unique and independent tadpole community (see more details in Data Analysis section).

For tadpole collection, two persons screened all available habitats in the waterbody for one hour (Heyer, 1976). Sweepings were systematized, considering the whole perimeter of the ponds' margins (Vasconcelos and Rossa-Feres, 2005; Santos et al., 2007; Both et al., 2009; Bolzan et al., 2016; Marques et al., 2018) and, for streams, we additionally used smaller dip nets that allowed us to screen smaller spaces between rocks (adapted from Jordani et al., 2017). During the sampling, we conducted a rigorous inspection of the presence of dead tadpoles and, among the collected tadpoles, we examined the presence of lethargy, which may be indicative of disease (Berger et al., 1998; Miller et al., 2015). Afterwards, the tadpoles were euthanized by immersion in a solution of 2% lidocaine (according to the Conselho Nacional para o Controle de Experimentação Animal - CONCEA, 2018) and later transferred to absolute ethanol (see Lambertini et al., 2013).

Analysis of the presence of depigmentation and zoosporangia

We conducted the taxonomic identification of the specimens using a stereomicroscope Yaxum/IX-AK02 and identification keys (e.g., Machado and Maltchik, 2007; Gonçalves, 2014). Concurrently, we classified the tadpoles according to the stage of ontogenetic development following Gosner (1960). We selected 899 tadpoles from stages 26 to 40 for histological and visual analysis of *Bd* (following Altig and Johnston, 1986). The selection criterion also considered the occurrence of the same species in more than three waterbodies to avoid species with a low frequency of occurrence.

To investigate the presence of *Bd* on the tadpoles, we combined macroscopic and microscopic inspections (Neves-da-Silva et al., 2021). Macroscopic inspection was made with a stereomicroscope (Yaxum/IX-AK02) to detect any sign of depigmentation in the oral apparatus (Lambertini et al. 2013; Supplementary Materials. Fig. S1). Depigmentation in the tooth rows and the corneous beak is directly associated with the presence of *Bd* and is promoted by an advanced stage of keratin loss generated by the activity of the fungus (Rachowicz and Vredenburg, 2004; Vieira et al., 2013). Therefore, the presence of depigmentation has been used as an indicator of the presence of *Bd* on the tadpoles (see Vieira et al., 2013; Valencia-Aguilar et al., 2016; Carvalho et al., 2017; Ruggeri et al., 2020; Neves-da-Silva et al., 2021).

Microscopic analyses were performed by inspecting tissue samples from the mouths of tadpoles. We sectioned the oral apparatus of each tadpole to obtain tissue samples of about 2 mm. The presence of *Bd* was evaluated by the detection of zoosporangia in the tissue samples after a detailed inspection in an optical microscope with a magnification of up to 400x for about 30 min (Vieira et al. 2013). All analyses (macro and microscopic) were conducted by the same person for standardization.

Ranavirus analysis

We extracted tissue samples from 1440 tadpoles of 24 waterbodies, which were analysed for the presence of *Ranavirus* spp. Only waterbodies with >50 sampled tadpoles were selected to ensure a sample size that is compatible with what is suggested for studies of viral infections (see DiGiacomo et al., 1986). We performed a pool of tissues per waterbody, totalling 24 samples. For that, we separated each tadpole's liver, gills, and kidneys using a surgical scalpel and dissecting needle under the stereomicroscope, cut the tissues (liver, gills, and kidneys) into small fragments, and stored the samples in individual 1.5-ml microtubes with absolute ethanol. We then put up to 25 mg of the tissue pool from tadpoles per waterbody in 1.5-ml microtubes with absolute ethanol to proceed with DNA extraction using the DNeasy® Blood & Tissue kit following the manufacturer's protocol (24 microtubes in total). After DNA extraction, we measured the concentration of the samples using NanoDrop Lite (Thermofisher) and diluted all samples to 20 ng/µl.

We used PCR analysis to detect the presence of the virus in each waterbody. We used specific primers for the genes MCP and Pol II, as described in Mazzoni et al. (2009), and determined the optimal annealing temperature for these primers by gradient PCR (from 50 to 65 °C). For each reaction, we used 12.5 µl of GoTaq® Colorless Master Mix1 µl of each starter at 10 µM, 5.0 µl of DNA template (100 ng.µl^{-1}) and 5.5 µl of ultrapure water to complete 25 µl for each reaction. We adapted the amplification conditions for each set of primers (Mazzoni et al., 2009). Therefore, we performed an initial 2-min cycle at 95 °C, followed by 60 seconds at 95 °C (denaturation), 60 seconds at 60 °C (annealing), and 90 seconds at 72 °C (MCP extension) or 30 seconds at 72 °C (POL-II extension), followed by a final extension of 5 min at 72 °C. The products of the amplification were transferred to agarose gel at 1%, dipped into TAE buffer solution (tris-acetate 40 mM, EDTA 1 mM), stained with 0.5 µg.ml^{-1} of ethidium bromide, and visualized with UV transilluminator.

Microhabitat and land use analysis

We evaluated the canopy cover surrounding each water body using a spherical lens (Universal clip lens; 180º) coupled to a cell phone (Xiaomi MI). The images were taken at the central point of the tadpole-collection site and later treated in the GapLight program, version 2.0 (Frazer et al., 1999) and presented as a percentage. We made a single measurement per waterbody (see Supplementary Materials, Table S2). The relevance of the canopy cover was based on studies showing that it affects the availability of food (periphyton; Skelly et al., 2002) and, consequently, the diversity of tadpoles (Jithin et al., 2022). For a broader range of evaluation, we described the land use in which waterbodies are located. Analyses were based on satellite images (Landsat 8 multispectral images, sensor Operational Land Imager from the U.S. Geological Survey; https://earthexplorer. usgs.gov) captured between January and July 2019. We selected images with minimal cloud cover and without significant radiometric noise. We performed the following pre-processing steps of the images: 1) geometric corrections, due to the inherent geometric distortions in images collected at different times, through the georeferencing of these images; 2) atmospheric corrections to reduce the interference of atmospheric scattering on the images (Soares et al., 2015); and 3) mosaic and enhancement of the different images in each season to reduce possible seasonal effects on the visual aspect of the images. Pre-processing was conducted using software ENVI, version 5.51 (L3Harris Geospatial, Boulder, Colorado, USA). After the pre-processing stages, we defined the classes of land use and occupation from observations in loco, considering the predominant use of the areas. The categories were established as Agriculture, Aquatic environments, Forest, Pastures, and Urbanization (Supplementary Material, Table S2). We classified the images based on their vectorization in ArcGIS software version 10.3 (Environmental Systems Research Institute Esri, Redlands, California, USA), considering the buffer with a 500-m radius for each waterbody. The buffer size was based on previous studies that describe the average habitat size for amphibians, ranging from 290 m (Semlitsch and Bodie, 2003) to 500 m (Canessa and Parris 2013). We consider the central point of the buffer to be the pond or stream (collection points). The polygons for each type of cover were reprojected to the Geocentric Reference System for the Americas (SIRGAS 2000), Universal Transverse Mercator (UTM) projection, zone 22S, and we calculated the areas in km^2.

Data Analysis

To test the existence of significant correlations between the presence of oral depigmentation and the presence of zoosporangia, we performed a Pearson correlation (r) using the “cor.test” function of the “vegan” package in the software R v.3.6.0 (R Core Team, 2021). We used linear mixed-effects models (LMMs) to evaluate the influence of the landscape variables (Supplementary information Table S2) on *Bd* prevalence in tadpoles. This analysis allows us to test the contribution of fixed and random effects in the distribution of observed data. We included the landscape variables as fixed effects (predictor) variables and the communities as random effects variables. We evaluated the significance of each explanatory and exploratory variable for *Bd* prevalence using the “ANOVA” function. We built several models with different sets of landscape descriptors so that all possible combinations could be evaluated. Model selection was done using the Akaike Information Criteria correcting (AICc) to select the model containing the most information among all candidate hypotheses (Buham and Anderson, 2002). We also considered the AICc weights (w), which are indicative of the empirical support for each model relative to others in the candidate set. Finally, we applied a threshold of AICc 2 units to define model support (in other words, we considered models with ΔAIC < 2 as equivalent; Zuur et al., 2009). GLMMs were built using the 'nlme', 'MuMIn' and 'lme4' packages in R software (Bates et al., 2015). We analysed all the full models using the "lme" function of the "lme4" package.

To list the anuran species with the potential to indicate the presence of *Bd*, we conducted an indicator species analysis using the "indicspecies" package in R (Cáceres and Legendre, 2009). As the first step, we used the function "pruneindicators" to determine the best set of indicator species. This analysis works in stages. First, it selects the species with positive values of prediction, sensitivity, and indication. Then, a "speciescomb" object with confidence intervals is produced, and the lower limits are used to select "indicator species", excluding species that show an occurrence pattern that is nested with other species. Finally, the function evaluates the coverage of the remaining set of species until the maximum indication values are reached to discriminate species with real indication potential (Cáceres and Legendre, 2009). Following this step, we used the "predict.indicators" function to determine the existence of communities (waterbodies) that would be considered indicators of the presence of *Bd*. This function calculates the probabilities corresponding to the communities to construct the indicator object. We used the function "pruneindicators" to find the communities with the highest indicator values. The groups are defined by species, which act as group indicators. The main indicators are those found in only one group (specificity) and, if all members of the group are positive for *Bd* (coverage), this species has "1" as its indication value. The specificity of a species is calculated considering the prevalence of the fungus and based on the number of individuals, while coverage only considers the fungus' presence or absence. Finally, we performed a principal components analysis (PCA) to observe the association between landscape classes and *Bd* prevalence in the tadpole communities using the "prcomp" function in the package vegan of R software.

Table 1. Anuran species sampled in 26 waterbodies (streams and ponds) in forest remnants from October 2018 to March 2019 in southern Brazil.

	Waterbodies																									
Family/Species	1	3	4	5	7	8	9	10	11	12	13	14	15	16	17	19	20	21	22	23	24	26	27	28	29	30
HYLIDAE																										
Boana curupi (Garcia, Faivovich and Haddad 2007)	0	0	0	0	0	0	0	0	0	0	0	0	49	34	0	0	0	32	0	20	40	0	0	0	0	0
Boana faber (Wied-Neuwied, 1821)	0	0	0	0	0	3	2	26	1	0	0	40	1	0	11	5	0	0	6	0	0	34	0	0	0	0
Dendropsophus microps (Peters, 1872)	0	0	0	11	0	0	0	0	4	0	2	0	0	0	0	0	0	0	18	0	0	5	0	0	0	0
Dendropsophus minutus (Peters, 1872)	5	0	0	0	0	10	0	0	0	5	7	0	1	0	0	34	2	0	0	0	0	0	0	0	0	0
Scinax fuscovarius (Lutz, 1925)	0	0	2	0	0	20	0	0	0	0	5	0	0	0	0	6	0	0	2	0	0	0	0	0	0	0
Scinax granulatus (Peters, 1871)	0	2	0	0	0	3	0	0	0	4	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Scinax perereca Pombal, Haddad and Kasahara, 1995	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	8	0	0	0	0	40	40	0
HYLODIDAE																										
Crossodactylus schmidti Gallardo, 1961	0	0	0	0	43	0	29	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	40	0	0	40
LEPTODACTYLIDAE																										
Physalaemus cuvieri Fitzinger, 1826	6	0	1	29	0	0	0	0	0	36	25	0	0	0	0	6	38	0	3	0	0	0	0	0	0	0
PHYLLOMEDUSIDAE																										
Phyllomedusa tetraploidea Pombal and Haddad, 1992	0	0	37	0	0	9	0	0	0	0	0	0	6	0	0	0	0	0	3	0	0	1	0	0	0	0
Total of individuals sampled by waterbody	11	2	40	40	43	45	31	26	7	40	40	40	59	34	11	50	40	40	40	20	40	40	40	40	40	40
Total of individuals sampled for *Bd* evaluation																										899

Table 2. Number of tadpoles sampled per waterbody in forest remnants in southern Brazil, from October 2018 to March 2019. Number of tadpoles positive for *Bd* and *Bd* prevalence as a percentage.

Waterbody	**Sample size**	***Bd*_positive**	***Bd*_prevalence %**
1	11	6	54.55
3	2	0	0.00
4	40	10	25.00
5	40	11	27.50
7	43	14	32.56
8	45	16	35.56
9	31	10	32.26
10	26	13	50.00
11	7	1	14.29
12	40	19	47.50
13	40	5	12.50
14	40	24	60.00
15	59	20	33.90
16	34	14	41.18
17	11	3	27.27
19	50	12	24.00
20	40	20	50.00
21	40	18	45.00
22	40	20	50.00
23	20	6	30.00
24	40	12	30.00
26	40	9	22.50
27	40	9	22.50
28	40	7	17.50
29	40	7	17.50
30	40	12	30.00
Sum	**899**	**298**	
Minimum	2.00	0.00	0.00
Maximum	59.00	24.00	60.00
Standard deviation	13.65	6.26	14.50

Mean	**34.57**	**11.46**	**32.04**

Results

The 10 analysed anuran species (Table 1) belong to four families: Hylidae (*Boana curupi*, *B. faber*, *Dendropsophus microps*, *D. minutus*, *Scinax fuscovarius*, *Scinax granulatus*, *S. perereca*), Hylodidae (*Crossodactylus schmidti*), Leptodactylidae (*Physalaemus cuvieri*), and Phyllomedusidae (*Phyllomedusa tetraploidea*). We recorded the depigmentation in the oral apparatus and/or *Bd* zoosporangia in tadpoles from 96.2% (25/26) of the communities. Of the 899 tadpoles analysed, 455 (50.6% of total) had depigmentation in the oral apparatus, 298 (33.1% of the total) had zoosporangia and 194 (21.6% of total) had both depigmentation and zoosporangia. We found a low correlation between the presence of depigmentation in the oral apparatus (n = 455) and the presence of zoosporangia (n = 298) ($r = 0.20$, $df = 897$, $p < 0.005$). The mean prevalence of *Bd* in the communities was 32% ± 14.5% (range, 0–60%) (Table 2). We did not find dead or lethargic tadpoles in any of the 26 communities analysed.

The LMM showed that there is a weak and non-significant influence of land use on *Bd* prevalence (contribution of fixed effects, $R^2m = 0.10$; contribution of fixed effects + random effects, $R^2c = 0.89$; Table S4). However, the analysis of indicator communities identified five tadpole communities as indicators of *Bd* presence (communities 15, 16, 21, 23, and 24; Table 3), which were explained by larger forested areas (Fig. 2). Of the 10 analysed species, three were indicators of the presence of *Bd* (Table 4). *Boana curupi* (Forest Treefrog) and *Crossodactylus schmidti* (Schmidt's Spinythumb Frog) had the greatest indicator values (specificity values close to 1). *Boana faber* (Gladiator Treefrog) was also selected as an indicator, but this species had values of specificity lower than *Boana curupi* and *Crossodactylus schmidti* (specificity value = 0.89). Finally, none of the three species showed exclusivity in *Bd* infection (sqrtIV = 0.54, *B. faber*; sqrtIV = 0.53, *B. curupi*; sqrtIV = 0.49, *C. schmidti*). All analysed tadpoles tested negative for ranavirus.

Fig. 2. Principal Component Analysis (PCA) showing the association between landscape classes and *Bd* prevalence in the tadpole communities from waterbodies in reproductive sites of Atlantic Forest habitats in southern Brazil from October 2018 to March 2019.

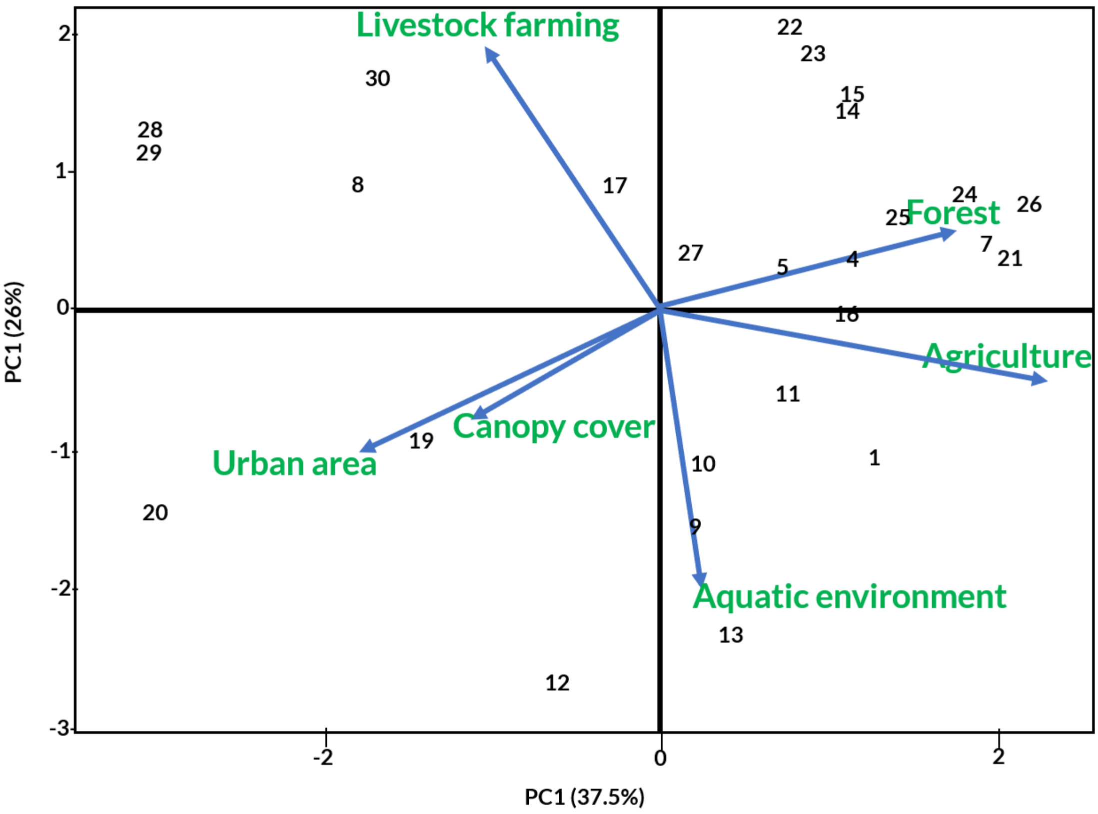

Table 3. Group of *Bd*-indicator waterbodies (communities) in forest remnants of southern Brazil. Abbreviation: Prob = Probability; Prob_CV = Probability coefficient of variation.

Waterbody code	Group	Prob	Prob_CV
1	0.00	0.00	0.00
2	0.00	0.00	0.00
4	0.00	0.00	0.00
5	0.00	0.00	0.00
6	0.00	0.00	0.00
7	0.00	0.00	0.00
8	0.00	0.99	1.00
9	0.00	0.00	0.00
10	0.00	0.00	0.00
11	0.00	0.00	0.00
12	0.00	0.00	0.00
13	0.00	0.00	0.00
14	0.00	0.00	0.00

15	1.00	0.99	0.99
16	1.00	0.99	0.99
17	0.00	0.00	0.00
18	0.00	0.00	0.00
19	0.00	0.00	0.00
20	0.00	0.00	0.00
21	1.00	1.00	0.00
22	0.00	0.00	0.00
23	1.00	0.99	0.99
24	1.00	0.99	0.99
26	0.00	0.00	0.00
27	0.00	0.00	0.00
28	0.00	0.00	0.00
29	0.00	0.00	0.00
30	0.00	0.00	0.00

Table 4. *Bd*-indicator species in tadpole communities from waterbodies in reproductive sites of Atlantic Forest habitats in southern Brazil from October 2018 to March 2019. A (specificity) = measurement of the species' specificity; B (coverage) = measurement of the species' coverage; sqrtIV = indicator value that considers both A and B and varies between 0 (*Bd* not present in any individual of this species) to 1 (*Bd* present in only this species and present in all individuals).

Species	A (specificity)	B (coverage)	sqrtIV	*p*-value
Boana faber	0.89	0.33	0.54	0.26
Boana curupi	1.00	0.29	0.53	0.14
Crossodactylus schmidti	1.00	0.24	0.49	0.21

Discussion

We did not detect ranavirus infection in the studied communities. However, ranavirus has already been recorded in tadpole populations about 200 km from our study area (Ruggeri et al., 2019). Thus, we argue that ranavirus is a potential threat to our populations. On the other hand, we found that more than 90% of the studied communities presented tadpoles infected with *Batrachochytrium dendrobatidis* (*Bd*). Among these communities, half of the tadpoles had depigmentation in the oral apparatus and more than

30% had zoosporangia. This result indicates a higher *Bd* prevalence than that previously observed in adults (see Ernetti et al., 2020; Santos et al., 2020; Delazeri et al., 2021).

Although tadpoles from communities in the southern and southeastern portions of the Atlantic Forest are often *Bd* infected, we recorded higher values of both prevalence and loads in southern Brazil (present study; Vieira et al., 2013; Carvalho et al., 2017; Ruggeri et al., 2018, 2020; Neves-da-Silva et al., 2021). At the same time, communities from the northeastern Atlantic Forest have lower infection levels (Lambertini et al., 2021; Belasen et al., 2022). This indicates a latitudinal variation in the suitability of tadpoles to *Bd* infection. Studies showed that *Bd* infection is favoured by colder climate regimes, which could be the reason for the higher prevalence and loads observed in southern Brazil (areas under a subtropical climate regime). Additionally, all species inspected for *Bd* in southern Brazil had infected individuals (Preuss et al. 2016; Ernetti et al., 2020; Santos et al., 2020; Delazeri et al., 2021). These results reinforce the hypothesis of a latitudinal component driving anuran suitability for *Bd* infections. This hypothesis deserves focused experiments for elucidation, which are not available until now. As we did not record dead or lethargic tadpoles, we propose another working hypothesis for future studies: the existence of tolerance to *Bd* in the evaluated tadpole communities. Once an indication of tolerance is detected, we can argue that tadpoles are acting as a *Bd* reservoir, as already reported in other studies (Valencia-Aguilar et al., 2016; Neves-da-Silva et al., 2021).

We did not find correlations between land use and *Bd* prevalence. Although the composition of anuran communities in the study area is affected by the configuration of the landscape (Santos et al., 2021), human changes in the landscape do not seem to affect the suitability of tadpoles for *Bd* infection. In fact, based on our evaluation of *Bd*-indicator communities, *Bd* was more associated with communities from larger forest areas. A possible explanation for the forest-*Bd* association is that the forested habitats are more shaded and generate microclimate conditions (such as lower temperatures) that favour *Bd* development (Beyer et al., 2015; Muletz-Wolz et al., 2019; Wanderley et al., 2019). As a result, we are more prone to find higher zoospore loads in amphibians from more forested areas (Delazeri et al., 2021; Lambertini et al., 2021). Our data highlight the risk to which tadpole communities are exposed, even those living in large forest patches (see Herczeg et al., 2021). Recently, the *Bd* suitability to infect anurans in forests has been associated with a genetically driven decrease in immune resistance among forest specialist anurans when their habitat is disturbed (Belasen et al., 2022). As we can see, the relationship between *Bd* infection and forest is a complex issue, but we argue that it must be investigated under a scenario of individual responses to landscape/habitat changes (Pereira da Silva et al., 2022).

As recorded in previous studies, we found a weak correlation between depigmentation in the oral apparatus and the presence of zoosporangia in tadpoles (Navarro-Lozzano et al., 2018). However, one must consider that the absence of depigmentation is not indicative of a *Bd*-free individual. Thus, we reinforce that *Bd* evaluation through oral depigmentation in the oral apparatus must be complemented with other techniques, such as histological analysis, qPCR, or histochemistry. *Bd* prevalence varied between communities, which could be explained by the differences in species composition among communities. Species would vary their response to *Bd* infection due to differences in aspects of their life history (Corey and Waite, 2008) and their reproduction mode (Lips et al., 2003). We analysed 10 anuran species from one of the richest communities ever evaluated (see Ruggeri et al., 2020; Neves-da-Silva et al., 2021). As more species are evaluated simultaneously, the more variable the results tend to be, making it difficult to find a general pattern at a community level. An alternative approach would be an evaluation based on functional diversity instead of taxonomical comparisons (Santos et al., in preparation; Wu et al., 2018).

Our results pointed out that *Bd*-indicator communities are represented mainly by forest-specialist species, such as *Boana curupi* and *Crossodactylus schmidti*. These species are also *Bd* indicators, which is of great concern since they are threatened and endemic species from southern Brazil and Argentina (CONSEMA, 2011; FZRGS, 2014; Frost, 2021; IUCN, 2021). Previous studies in this region already indicated *B. curupi* and *C. schmidti* as species that are vulnerable to *Bd*, mainly *B. curupi*, which showed high prevalence and zoospore loads in adults (Preuss et al., 2016; Forti et al., 2020; Delazeri et al., 2021). Another *Bd* indicator was *Boana faber*. This is a species of particular interest because, differently from *B. curupi* and *C. schmidti*, females of *B. faber* lay their eggs in ponds in open areas, not forests (Haddad et al., 2013; Oliveira et al., 2016; Frost, 2021). This result does not weaken our hypothesis of a high association between forest habitats and *Bd* infections since tadpoles of *B. faber* have a long period of larval development (8 to 12 months; Martins, 1993; Madalozzo et al., 2013). Longer development cycles increase their exposure time to *Bd zoospores* (Bradley et al., 2019; Ruggeri et al., 2020; Neves-da-Silva et al., 2021) leading to a higher prevalence of fungal infections (Neves-da-Silva et al., 2021). Although a little speculative, this relationship reinforces the relevance of the natural history components on anuran suitability to *Bd* infection (Alvarado-Rybak et al., 2021).

We must consider that our study site is a highly fragmented landscape formed by forest patches of different sizes. Even the larger patches were formed because of an intense fragmentation/deforestation process driven by human activities (agriculture, pastures, urbanization; Wanderley et al., 2019; Ernetti et al., 2020; Preuss et al., 2020). The observed association between forest and *Bd* highlights that even communities from the last preserved forests (patches) are not free of threats to their survival. Regarding this, we consider that the use of *Bd*-indicator species and *Bd*-indicator communities is a useful approach for conservational and landscape-management proposals.

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References

Altig, R., Johnston, G.F., 1986. Major characteristics of free-living anuran tadpoles. Smithson. Herpetol. Inf. Serv., 67, 1–75.

Altizer, S., Ostfeld, R.S., Johnson, P.T.J., Kutz, S., Harvell, C.D., 2013. Climate change and infectious diseases: from evidence to a predictive framework. Science, 341, 514–519. https://doi.org/10.1126/science.1239401

Alvarado-Rybak, M., Lepe-Lopez, M., Peñafiel-Ricaurte, A. et al., 2021. Bioclimatic and anthropogenic variables shape the occurrence of *Batrachochytrium dendrobatidis* over a large latitudinal gradient. Sci. Rep., 11, 17383. https://doi.org/10.1038/s41598-021-96535-w.

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L.M., Sparovek, G., 2013. Köppen's climate classification map for Brazil. Meteorol. Zeitschrift, 22, 711–728. https://dx.doi.org/10.1127/0941-2948/2013/0507

Bach, N.C., Marino, D.J.G., Natale, G.S., Somoza, G.M., 2018. Effects of glyphosate and its commercial formulation, Roundup ® Ultramax, on liver histology of tadpoles of the neotropical frog, *Leptodactylus latrans* (amphibia: Anura). Chemosphere, 202, 289–297. https://doi.org/10.1016/j.chemosphere.2018.03.110

Belasen, A.M., Amses, K.R., Clemons, R.A., Becker, G., Toledo, F., James, T., 2022. Habitat fragmentation in the Brazilian Atlantic Forest is associated with erosion of frog immunogenetic diversity and increased fungal infections. Immunogenetics, https://doi.org/10.1007/s00251-022-01252-x

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw., 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Becker, C.G., Fonseca, C.R., Haddad, C.F.B., Prado, P.I., 2010. Habitat split as a cause of local population declines of amphibians with aquatic larvae. Cons. Biol., 24(1), 287–294. https://doi.org/10.1111/j.1523-1739.2009.01324.x

Becker, M.H., Walke, J.B., Murrill, L., Woodhams, D.C., Reinert, L.K., Rollins-Smith, L.A., Burzynski, E.A. Umile TP, Minbiole KP, Belden LK., 2015. Phylogenetic distribution of symbiotic bacteria from Panamanian amphibians that inhibit growth of the lethal fungal pathogen *Batrachochytrium dendrobatidis*. Mol. Ecol., 24(7), 1628–41. https://doi.org/10.1111/mec.13135

Becker, C.G., Zamudio, K.R., 2011. Tropical amphibian populations experience higher disease risk in natural habitats. PNAS, 108, 9893–9898. https://doi.org/10.1073/pnas.1014497108

Berger, L., Hyatt, A.D., Speare. R., Longcore, J.E., 2005. Life cycle stages of the amphibian chytrid *Batrachchytrium dendrobatidis*. Dis. Aquat. Org., 68, 51– 63.

Beyer, S.E., Phillips, C.A., Schooley. R.L., 2015. Canopy cover and drought influence the landscape epidemiology of an amphibian chytrid fungus. Ecosphere, 6(5):78. http://dx.doi.org/10.1890/ES14- 00263.1.

Blaustein, A., Romansic, J.M., Schessele, E.A., Han, B.A., Pessier, A.P., Longcore, J.E., 2005. Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. Conserv. Biol., 19, 1460–1468.

Bolzan, A.M.R., Saccol, S.A., Santos, T.G., 2016. Composition and diversity of anurans in the largest conservation unit in Pampa biome, Brazil. Biota Neotrop., 16(2), e20150113. http://doi.org/10.1590/16760611-BN-2015-0113

Both, C., Sole, M., Santos, T.G., Cechin, S.Z., 2009. The role of spatial and temporal descriptors for neotropical tadpole communities in southern Brazil. Hydrobiologia, 624, 125–138. http://doi.org/10.1007/s10750-008-9685-5

Bradley, P.W., Brawner, M.D., Raffel, T.R., Rohr, J.R., Olson, D.H., Blaustein, A.R., 2019. Shifts in temperature influence how *Batrachochytrium dendrobatidis* infects amphibian larvae. PLoS One, 14(9), e0222237. https://doi.org/10.1371/journal.pone.0222237

Cáceres, M. de, Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. Ecology, 90, 3566–3574.

Canessa, S., Parris, K.M., 2013. Multi-scale, direct and indirect effects of the urban stream syndrome on amphibian communities in streams. PLoS ONE, 8, e70262. https://doi.org/10.1371/journal.pone.0070262

Carvalho, T., Becker, C.G., Toledo, L.F., 2017. Historical amphibian declines and extinctions in Brazil linked to chytridiomycosis. Proc. R. Soc. London Ser. B: Biol. Sc., 28, 20162254. https://doi.org/10.1098/rspb.2016.2254

Chinchar, V.G., Hick, P., Ince, I.A., Jancovich, J.K., Marschang, R., Qin, Q., Subramaniam, K., Waltzek, T.B., Whittington, R., Williams, T., Zhang, Q., ICTV., 2017. Report Consortium, ICTV virus taxonomy profiles: Iridoviridae. J. Gen. Virol., 98, 890–891. https://doi.org/10.1099/jgv.0.000818.

Conselho Nacional para o Controle de Experimentação Animal (CONCEA). 2018. Normative Resolution nº 37. National Council for Animal Experimentation Euthanasia Practice Directive. Official Diary, Brasília.

CONSEMA – Conselho Estadual do Meio Ambiente, 2011. Lista de Espécies da Fauna Ameaçadas de Extinção no Estado de Santa Catarina. https://www.ima.sc.gov.br/index.php/biodiversidade/biodiversidade/fauna (acessed 30 January 2022).

Corey, S.J., Waite, T.A., 2008. Phylogenetic autocorrelation of extinction threat in globally imperilled amphibians. Divers. Distrib., 14(4), 614–629. https://doi.org/10.1111/j.1472-4642.2007.00448.x

Daszak, P., Berger, L., Cunningham, A.A., Hyatt, A.D., Green, D., Speare, R., 1999. Emerging infectious diseases and amphibian population declines. Emerg. Infect. Dis., 5, 1–23.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2003. Infectious disease and amphibian population declines. Divers. Distrib., 9, 141–150.

DiGiacomo, R.F., Koepsell, T.D., 1986. Sampling for detection of infection or disease in animal populations. J. Am. Vet. Med. Assoc., 189, 22–23.

Delazeri, F., Ernetti, J.R., De Bastiani, V.I.M., Lingnau, R., Toledo, L.F., Lucas, E.M., 2021. Forest cover influences chytrid infections in populations of *Boana curupi*, a threatened treefrog of south Brazil. Dis. Aquat. Org., 144, 133–142. https://doi.org/10.3354/dao03585

Duffus, A.L.J., Waltzek, T.B., Stöhr, A.C., Allender, M.C., Gotesman, M., Whittington, R.J., Hick, P., Hines, M.K., Marschang, R.E., 2015. Distribution and host range of ranaviruses. In: Gray, M.J., Chinchar, V.G. (Eds.) Ranaviruses: lethal pathogens of ectothermic vertebrates, Springer, New York, pp. 9-57.

Ernetti, J.R., Boschetti, J.P., Delazeri. F., De Bastiani, V.I.M., et al., 2020. High temporal and individual variation in the prevalence and intensity of chytrid infection in the southernmost leaf frog of the genus *Pithecopus* (Anura, Phyllomedusidae). Hydrobiologia, 847, 3355−3364. https://doi.org/10.1007/s10750-020-04339-2

Fisher, M.C., Garner, T.W.J., 2020. Chytrid fungi and global amphibian declines. Nat. Rev. Microbiol., 18, 332-343. https://doi.org/10.1038/s41579-020-0335-x.

Forti, L.R., Pontes, M.R., Alcantara, E.P., Morais, D.H., Da Silva, R.J., Dodonov, P., Toledo, L.F., 2020. Torrent frogs have fewer macroparasites but higher rates of chytrid infection in landscapes with smaller forest cover. Ecosphere, 11(6), e03169. https://doi.org/10.1002/ecs2.3169

Frazer, G.W., Canham, C.D., Lertzman, K.P., 1999. Manual do Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-color fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York.

Frost, D.R., 2021. Amphibian Species of the World: an Online Reference. Version 6.1. American Museum of Natural History, New York. https://doi.org/10.5531/db.vz.0001

FZRGS – Fundação Zoobotânica do Rio Grande do Sul, 2014. Lista de espécies da fauna gaúcha ameaçadas de extinção.

https://www.al.rs.gov.br/filerepository/replegis/arquivos/dec%2051.797.pdf (acessed 20 January 2022).

García-Feria, L.M., Brousset, D.M., Cervantes-Olivares, R.A., 2019. Factores abióticos y bióticos determinantes para la presencia de *Batrachochytrium dendrobatidis* en anfibios mexicanos. Acta. Zool. Mex. Nueva Ser., 35, 1–18. https://doi.org/10.21829/azm.2019.3502066.

Garner, T. W. J., Garcia, G., Carroll, B., Fisher, M.C., 2009. Using itraconazole to clear Batrachochytrium dendrobatidis infection, and subsequent depigmentation of Alytes muletensis tadpoles. Dis. Aquat. Org., 83, 257–260. https://doi.org/10.3354/dao02008

Gervasi, S.S., Urbina, J., Hua, J., Chestnut, T., Relyea, R.A., Blaustein, A.R., 2013. Experimental evidence for American bullfrog (*Lithobates catesbeianus*) susceptibility to chytrid fungus (*Batrachochytrium dendrobatidis*). EcoHealth, 10, 166–171. https://doi.org/10.1007/s10393-013-0832-8

Giam, X., 2017. Global biodiversity loss from tropical deforestation. PNAS, 114(23), 5775–5777. doi:10.1073/pnas.1706264114.

Gillespie, T.R, Chapman, C.A, Greiner, E.C., 2005. Effects of logging on gastrointestinal parasite infections and infection risk in African primates. J. Appl. Ecol., 42, 699–707. https://www.jstor.org/stable/3505903.

Gonçalves, D.D.S., 2014. Diversidade e chave de identificação para girinos ocorrentes em áreas de floresta com araucária. Ph.D. Dissertation, Universidade Federal do Paraná, Curitiba, Brasil. 131 p.

Gosner, K.L., 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica, 16, 183–190. https://www.jstor.org/stable/3890061

Green, D.E., Converse, K.A., Schrader, A.K., 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. Ann. N.Y. Acad. Sci., 969, 323–339. https://doi.org/10.1111/j.1749-6632.2002.tb04400.x

Gray, M.J., Chinchar, G.V., 2015. Ranaviruses: Lethal pathogens of ectothermic vertebrates. Springer, Berlin.

Haddad, C.F.B., Toledo, L.F., Prado, C.P.A., Loebmann, D., Gasparini, J.L., Sazima, I., 2013. Guide to the Amphibians of the Atlantic Forest: Diversity and Biology. Anolis Books, São Paulo.

Hanlon, S.M., Lynch, K.J., Kerby, J., Parris, M.J. 2015. *Batrachochytrium dendrobatidis* exposure effects on foraging efficiencies and body size in anuran tadpoles. Dis. Aq. Org., 112, 237–242.

Herczeg, D., Ujszegi, J., Kásler, A., Holly, D., Hettyey, A., 2021. Host–multiparasite interactions in amphibians: a review. Parasites Vectors, 14, 296. https://doi.org/10.1186/s13071-021-04796-1

Herek, J.S., Vargas, L., Trindade, S.A.R., Rutkoski, C.F., Macagnan, N., Hartmann, P.A., Hartmann, M.T., 2020. Can environmental concentrations of glyphosate affect survival and cause malformation in amphibians? Effects from a glyphosate-based herbicide on *Physalaemus cuvieri* and *P. gracilis* (Anura: Leptodactylidae). Environ. Sc. Pollut. Res., 27, 22619–22630. https://doi.org/10.1007/s11356-020-08869-z

Hernández-López, P., Santiago-Alarcón, D., López-Velázquez, A., 2018. Factores ecológicos que afectan la infección por *Batrachochytrium dendrobatidis* en anfibios de México, in: López-Velázquez, A., Basanta, M.D., Ochoa-Ochoa, L.M. (Eds.). Quitridiomicosis en México, Publicaciones de la Sociedad Herpetológica Mexicana, pp. 53–78.

Heyer, W.R., 1976. Studies on larval amphibian habitat partitioning. Smithson. Contr. Zool., 242, 1–27. https://doi.org/10.5479/si.00810282.242.

Hoverman, J.T., Gray, M.J., Haislip, J.T., Miller, D.L., 2011., Phylogeny, life history, and ecology contribute to differences in amphibian susceptibility to ranaviruses. EcoHealth, 8(3), 301–19. https://doi.org/10.1007/s10393-011-0717-7

IAP – Instituto Ambiental do Paraná. 2004. Plano de Manejo do Parque Estadual de Vila Velha. Curitiba. http://www.iap.pr.gov.br/pagina-1255.html (accessed 20 May 2020).

IUCN – International Union for the Conservation of Nature. 2021., IUCN Red List of Threatened Species. https://www.iucnredlist.org

Jancovich, J.K., Steckler, N., Waltzek, T.B., 2015. Ranavirus taxonomy and phylogeny. In: Gray, M.J., Chinchar, V.G. (eds) Ranaviruses: lethal pathogens of ectothermic vertebrates. Springer, NewYork, pp. 59-70.

Jenkinson, T.S., Betancourt Román, C.M., Lambertini, C., Valencia-Aguilar, A., Rodriguez, D., Nunes-de-Almeida, C.H., Ruggeri, J., Belasen, A.M., da Silva Leite, D., Zamudio, K.R., Longcore, J.E., Toledo, F.L., James, T.Y. 2016. Amphibian-killing chytrid in Brazil comprises both locally endemic and globally expanding populations. Mol. Ecol., 25(13), 2978-96. doi: 10.1111/mec.13599

Jithin, V., Jeyaraj, J., Abhijit, D. 2022. Influence of Check Dams on the Activity Pattern and Morphometric Traits of Overwintering Tadpoles in the Western Himalaya. Limnologica, 95. 125992. 10.1016/j.limno.2022.125992.

Jones, K.E, Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L, Daszak, P., 2008. Global trends in emerging infectious diseases. Nature, 451(21), 990–994. https://doi.org/10.1038/nature06536

Jordani, M.X., Ouchi-De-Melo, L.S., Queiroz, C.S., Rossa-Feres, D.C., Garey, M.V., 2017. Tadpole community structure in lentic and lotic habitats: richness and diversity in the Atlantic Rainforest lowland. Herpetol. J., 27, 299–306.

Kärvemo, S., Laurila, A., Höglund, J., 2019. Urban environment and reservoir host species are associated with *Batrachochytrium dendrobatidis* infection prevalence in the common toad. Dis. Aquat. Organ., 11;134(1):33-42. https://doi.org/10.3354/dao03359.

Kärvemo, S., Meurling, S., Berger, D., Höglund, J., Laurila, A., 2018. Effects of host species and environmental factors on the prevalence of *Batrachochytrium dendrobatidis* in northern Europe. PLoS ONE, 13(10), e0199852. https://doi.org/10.1371/journal.pone.0199852

Knapp, R.A., Morgan, J.A.T., 2006. Tadpole Mouthpart Depigmentation as an Accurate Indicator of Chytridiomycosis, an Emerging Disease of Amphibians. Copeia, 2, 188-197. https://doi.org/10.1643/0045-8511

Lambertini, C., Rodrigues, D., Brito, F.B., Leite, D.S., Toledo, L.F., 2013. Diagnóstico do fungo quitrídio: *Batrachochytrium dendrobatidis*. Herpetol. Bras., 2, 12–17.

Lambertini, C., Becker, C.G., Belasen, A., Valencia-Aguilar, A., et al., 2021. Biotic and abiotic determinants of *Batrachochytrium dendrobatidis* infections in amphibians of the Brazilian Atlantic Forest. Fungal Ecol., 49, 100995. https://doi.org/10.1016/j.funeco.2020.100995.

Latney, L.V., Klaphake, E., 2020. Selected Emerging Infectious Diseases of Amphibians. Vet. Clin. North Am. Exot. Anim. Pract., 23(2), 397–412. https://10.1016/j.cvex.2020.01.003

Lips, K.R., Reeve, J.D., Witters, L.R., 2003. Ecological traits predicting amphibian population declines in Central America. Conserv. Biol., 17(4):1078–1088.

Longcore, J.E., Pessier, A.P., Nichols, D.K., 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a Chytrid Pathogenic to Amphibians. Mycologia. 91, 219–227.

Machado, I.F., Maltchik, L., 2007. Check-list of diversity of anurans in Rio Grande do Sul (Brazil) and a classification propose for larval forms. Neotrop. Biol. Conserv., 2(2), 101–116.

Madalozzo, B., Both, C., Cechin, S., 2013. Temporal distribution and age structure of tadpoles of *Hypsiboas faber* and *H. leptolineatus* in ponds: how do they coexist? J. Nat. Hist., 47, 2575−2581. https://doi.org/10.1080/00222933.2013.773100

Martel, A., Adriaensen, C., Sharifian-Fard, M., Slouijs, A.S.V.D., Louette, G., Baert, K., Crombaghs, B., Dewulf, J., Pasmans, F., 2013. The absence of zoonotic agents in invasive bullfrogs (*Lithobates catesbeianus*) in Belgium and the Netherlands. EcoHealth, 10, 344–347. https://doi.org/10.1007/s10393-013-0864-0

Martins, M., 1993. Observations on nest dynamics and embryonic and larval development in the nest building gladiator frog, *Hyla faber*. Amphibia-Reptilia, 14, 411−421.

Marques, N.C.S., Rattis, L., Nomura, F., 2018. Local environmental conditions affecting anuran tadpoles' microhabitat choice and morphological adaptation. Mar. Freshw. Res. doi:10.1071/MF18106.

Mazzoni, R., de Mesquita, A., Fleury, L., et al., 2009. Mass mortality associated with a frog virus 3- like Ranavirus infection in farmed tadpoles *Rana catesbeiana* from Brazil. Dis. Aquat. Org., 86, 181–191. https://doi.org/10.3354/dao02096.

McMillan, K.M., Lesbarrères, D., Harrison, X.A., Garner, T.W.J., 2020. Spatiotemporal heterogeneity decouples infection parameters of amphibian chytridiomycosis. J. Anim. Ecol., 89(4), 1109−1121. https://doi.org/10.1111/1365-2656.13170

Miller, D., Gray, M., Storfer, A., 2011. Ecopathology of ranaviruses infecting amphibians. Viruses, 3, 2351–2373.

Mittermeier, R.A., Fonseca, G.A.B., Rylands, A.B., Brandon, K., 2005. A brief history of biodiversity conservation in Brazil. Conserv. Biol., 19, 601–611. https://doi.org/10.1111/j.1523-1739.2005.00709.x

Mittermeier, R.A., Turner, W.R., Larsen, F.W., Brooks, T.M., Gascon, C., 2011. Global biodiversity conservation: the critical role of hotspots. In: Zachos, F.E., Habel, J.C. (Eds.), Biodiversity Hotspots. Springer, Berlin, Heidelberg, pp. 3–22. http://dx.doi.org/10.1007/978-3-642-20992-5_1

Muletz-Wolz, C.R., Barnett, S.E., DiRenzo, G.V., Zamudio, K.R., Toledo, L.F., James, T.Y., Lips, K.R., 2019. Diverse genotypes of the amphibian-killing fungus produce distinct phenotypes through plastic responses to temperature. J. Evolution Biol., 32, 287–298. https://doi.org/10.1111/jeb.13413

Narayan, E.J., Graham, C., McCallum, H., Hero, J-M., 2014. Over-Wintering Tadpoles of *Mixophyes fasciolatus* Act as Reservoir Host for *Batrachochytrium dendrobatidis*. PLoS ONE, 9(3), e92499. https://doi.org/10.1371/journal.pone.0092499

Navarro-Lozano, A, Sánchez-Domene, D., Rossa-Feres, D.C., Bosch, J., Sawaya, R.J., 2018. Are oral deformities in tadpoles accurate indicators of anuran chytridiomycosis? PLoS ONE, 13(1), e0190955. https://doi.org/10.1371/journal.pone.0190955

Neves-Da-Silva, D., Borges-Júnior, V.N.T., Branco, C.W.C., Carvalho-e-Silva, A.M.P.T., 2021. Effects of intrinsic and extrinsic factors on the prevalence of the fungus *Batrachochytrium dendrobatidis* (Chytridiomycota) in stream tadpoles in the Atlantic Forest domain. Aquat. Ecol., 55, 891–902. https://doi.org/10.1007/s10452-021-09869-y

Nichols, D.K., Lamirande, E.W., Pessier, A.P, Longcore, J.E., 2001. Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. J. Wildl. Dis., 37(1), 1–11. https://doi.org/10.7589/0090-3558-37.1.1

O'Hanlon, S.J., Rieux, A., Farrer, R.A., Rosa, G.M., Waldman, B., Bataille, A., Kosch, T.A., Murray, K.A., Brankovics, B., Fumagalli, M., et al., 2018. Recent Asian origin of chytrid fungi causing global amphibian declines. Science. 360, 621–627. https://doi.org/10.1126/science.aar1965

Oliveira, M., Aver, G.F., Moreira, L.F.B., Colombo, P., Tozetti, A.M. 2016. Daily Movement and Microhabitat Use by the Blacksmith Treefrog *Hypsiboas faber* (Anura: Hylidae) during the Breeding Season in a Subtemperate Forest of Southern Brazil. South American Journal of Herpetology 11, 89–97.

Olson, D.H., Aanensen, D.M., Ronnenberg, K.L., et al., 2013. Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. PLoS ONE, 8(2), e56802. https://doi.org/10.1371/journ al.pone.0056802

Pessier, A.P., Nichols, D.K., Longcore, J.E., Fuller, M.S., 1999. Cutaneous Chytridiomycosis in Poison Dart Frogs (*Dendrobates* spp.) and White's Tree Frogs (*Litoria Caerulea*). J. Vet. Diag. Invest., 11(2), 194–199. https://doi.org/10.1177/104063879901100219

Pereira da Silva, A.A., Franquelino, A.R., Teodoro, P.E., Montanari, R., Faria, G.A., Ribeiro da Silva, C.H., et al., 2022. The fewer, the better fare: Can the loss of vegetation in the Cerrado drive the increase in dengue fever cases infection? PLoS ONE, 17 (1), e0262473. https://doi.org/10.1371/journal.pone.0262473

Pillar, V.P., Vélez, E., 2010. Extinção dos Campos Sulinos em Unidades de Conservação: um Fenômeno Natural ou um Problema Ético? Nature Conserv, 8, 84–86.

Piotrowski, J.S., Annis, S.L., Longcore, J.E., 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia, 96, 9–15.

Piovia-Scott, J., Pope, K.L., Lawler, S.P, Cole, E.M., Foley, J.E., 2011. Factors related to the distribution and prevalence of the fungal pathogen *Batrachochytrium dendrobatidis* in *Rana cascadae* and other amphibians in the Klamath Mountains. Biol. Conserv., 144, 2913–2921. https://doi.org/10.1016/J.BIOCON.2011.08.008

Preuss, J.F., Lambertini, C., Leite, D.S., Toledo, L.F., Lucas, E.M., 2016. Crossing the threshold: an amphibian assemblage highly infected with *Batrachochytrium dendrobatidis* in the southern Brazilian Atlantic Forest. Stud. Neotrop. Fauna Environ., 51, 68−77.

Preuss, J.F.P., Greenspan, S.E., Rossi, E.M.; Gonsales, E.M.G., Neely, W.J., Valiati, V.H., Woodhams, D.C., Becker, C.G., Tozetti, A.M., 2020. Widespread pig farming practice linked to shifts in skin microbiomes and disease in pond-breeding amphibians. Environ. Sci. Technol., 54 (18), 11301−11312. https://doi.org/10.1021/ACS.EST.0C03219

Rachowicz, L.J., Vredenburg, V.T., 2004. Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. Dis. Aquat. Organ., 61(1-2), 75-83. https://doi.org/10.3354/dao061075

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Rijks, J.M., Saucedo, B., Spitzen-van der Sluijs, A., Wilkie, G.S., van Asten, A.J.A.M., van den Broek, J., et al., 2016. Investigation of Amphibian Mortality Events in Wildlife Reveals an On-Going Ranavirus Epidemic in the North of the Netherlands. PLoS ONE, 11(6), e0157473. https://doi.org/10.1371/journal.pone.0157473

Ribeiro, M.C., Metzger, J.P., Martensen, A.C., Ponzoni, F.J., Hirota, M.M., 2009. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biol. Conserv., 142, 1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021

Rosenblum, E.B., Stajich, J.E., Maddox, N., Eisen, M.B., 2008. Global gene expression profiles for life stages of the deadly amphibian pathogen *Batrachochytrium dendrobatidis*. PNAS, 105(44), 17034–17039. https://doi.org/10.1073/pnas.0804173105

Ruggeri, J., Toledo, L.F., Carvalho-e-Silva, S.P., 2018. Stream tadpoles present high prevalence but low infection loads of *Batrachochytrium dendrobatidis* (Chytridiomycota). Hydrobiologia, 806, 303–311. https://doi.org/10.1007/s10750-017-3367-0.

Ruggeri, J., Ribeiro, L.P., Pontes, M.R., Toffolo, C., Candido, M., Carriero, M.M., Zanella, N., Sousa, R.L.M., Toledo, L.F., 2019. Discovery of Wild Amphibians Infected with Ranavirus in Brazil. J. Wildl. Dis., 55(4), 897–902. https://doi.org/10.7589/2018-09-224

Ruggeri, J., Martins, A.G.D.S., Domingos, A.H.R., Santos, I., Viroomal, I.B., Toledo, L.F., 2020. Seasonal prevalence of the amphibian chytrid in a tropical pond-dwelling tadpole species. Dis. Aquat. Org., 142, 171–176. https://doi.org/10.3354/dao03539

Santa Catarina, 1986. Gabinete de Planejamento e Coordenação Geral. Atlas de Santa Catarina. Aerofoto Cruzeiro, Rio de Janeiro.

Santos, R.C., Bastiani, V.I.M., Medina, D., Ribeiro, L.P. et al., 2020. High prevalence and low intensity of infection by *Batrachochytrium dendrobatidis* in rainforest bullfrog populations in southern Brazil. Herpetol. Conserv. Biol., 15, 118−130.

Santos, R.C., Dalmolin, D.A., Brum, D., Veronez, M.R., Lucas, E.M., Tozetti, A.M., 2021. Association Between Land Use and Composition of Amphibian Species in Temperate Brazilian Forest Habitats. Herpetol. Conserv. Biol., 16(3), 612–623.

Santos, R.C., Brum, D., Dalmolin, D., Farina R.K., Lucas, E.M., Tozetti, A. M., in preparation. Trait-environment relationship in tadpoles of the southern Atlantic Forest. bioRxiv, Ecology. DOI: 10.1101/2021.10.08.463663

Santos, T.G., Rossa-Feres, D.C., Casatti, L., 2007. Diversidade e distribuição espaço-temporal de anuros em região com pronunciada estação seca no sudeste do Brasil. Iheringia, Série Zool., 97, 37–49.

Sapsford, S.J., Alford, R.A., Schwarzkopf, L., 2018. Disentangling causes of seasonal infection prevalence patterns: tropical tadpoles and chytridiomycosis as a model system. Dis. Aquat. Organ., 130, 83–93. https://doi.org/10.3354/dao03269

Scheele, B.C., Pasmans, F., Skerratt, L.F., Berger, L. et al., 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science, 363, 1459–1463. https://doi.org/10.1126/science.aav0379

Schock, D.M., Bollinger, T.K., Collins, J.P., 2010. Mortality Rates Differ among Amphibian Populations Exposed to Three Strains of a Lethal Ranavirus. EcoHealth, 6, 438–448.

Semlitsch, R.D., Bodie, J.R., 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. Conserv. Biol., 17, 1219–1228.

Simpkins, C. A., Kriger, K., Hero, J-M., 2017. Prevalence of *Batrachochytrium dendrobatidis* on amphibians from low pH, oligotrophic waterbodies. Herpetol. Rev., 48(4), 775-776.

Skelly, D., Freidenburg, L.K., Kiesecker, J., 2002. Forest Canopy and the Performance of Larval Amphibians. Ecology, 83, 983-992.

Soares, F.S., Almeida, R.K., Rubim, I.B., Barros, R.S., Cruz, C.B.M., Mello, G.V., Baptista Neto, J.A., 2015. Análise comparativa da correção atmosférica de imagem do Landsat 8: o uso do 6S e do ATCOR2. In: Simpósio Brasileiro de Sensoriamento Remoto. Gherardi, D.F.M., L.E.O.C. Aragão (Eds.). Instituto Nacional de Pesquisas Espaciais, João Pessoa, pp. 1821–1825.

Sonn, J.M., Berman, S., Richards-Zawacki, C.L., 2017. Influence of temperature on chytridiomycosis in vivo. EcoHealth, 14, 762–770. https://doi.org/10.1007/s10393-017-1269-2

SOS MATA ATLÂNTICA; INPE - Instituto Nacional de Pesquisas Espaciais, 2008. Atlas dos remanescentes florestais da Mata Atlântica, período de 2000 a 2005. https://www.sosmatatlantica.org.br (Accessed 20 February 2020)

Valencia-Aguilar, A., Toledo, L.F., Vital, M.V., Mott, T., 2016. Seasonality, environmental factors, and host behavior linked to disease risk in stream-dwelling tadpoles. Herpetologica, 72, 98–106. https://doi.org/10.1655/HERPETOLOGICA-D-15-00013

Vasconcelos, T.S., Rossa-Feres, D.C., 2005. Diversidade, distribuição espacial e temporal de anfíbios anuros (Amphibia, Anura) na região noroeste do Estado de São Paulo. Biota Neotrop, 5(2), 1–14. https://doi.org/10.1590/S1676-06032005000300010

Veloso, H.P., Goés-Filho, L., 1982. Fitogeografia brasileira: classificação fisionômico-ecológica da vegetação neotropical. Ministério das Minas e Energia-Secretaria Geral-Projeto RADAM BRASIL, Salvador.

Vieira, C.A., Toledo, L.F., Longcore, J.E., Longcore, J.R., 2013. Body length of *Hylodes* cf. *ornatus* and *Lithobates catesbeianus* tadpoles, depigmentation of mouthparts, and presence of *Batrachochytrium dendrobatidis* are related. Braz. J. Biol., 73, 195–199. https://doi.org/10.1590/S1519-69842013000100021

Wanderley, R.L.N., Domingues, L.M., Joly, C.A., Rocha, H.R., 2019. Relationship between land surface temperature and fraction of anthropized area in the Atlantic forest region, Brazil. PLoS ONE, 14(12), e0225443, 2019. https://doi.org/10.1371/journal.pone.0225443

Wu, N.C., Cramp, R.L., Franklin, C.E., 2018. Body size influences energetic and osmoregulatory costs in frogs infected with *Batrachochytrium dendrobatidis*. Sci. Rep., 8, 3739. https://doi.org/10.1038/s41598-018-22002-8

Supplementary Material

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Title: Effects of landscape on the incidence of infectious diseases (*Bd* and ranavirus) in tadpoles in the southern Atlantic Forest

Table S1. Description of the waterbodies (community) sampled in forest remnants in southern Brazil, from October 2018 to March 2019. VV = Parque Estadual de Vila Velha; RG = Parque Estadual Rio Guarani; RE = Reserva Privada Enele; PA = Parque Estadual das Araucárias; QQ = Reserva Privada Quebra-Queixo; FP = Parque Estadual Fritz Plaumann; PT = Parque Estadual do Turvo.

Waterbody	Study area	Remnant	State	Geographic Coordinates	Sample point	Description of the place
1	1	VV	PR	25°13'44.23"S 50° 2'8.87"O	Pond	Pond, with grassy and aquatic vegetation, in secondary forest and close to a road, inside protected area.
4	1	VV	PR	25°15'33.85"S 50° 1'26.77"O	Pond	Pond, with landscape of exposed soil, grass, and shrubs close to secondary vegetation, outside the protected area.
5	1	VV	PR	25°15'29.01"S 50° 1'20.75"O	Stream	Stream, with landscape of exposed soil, grass, and shrubs, close to secondary vegetation, outside the protected area.

7	2	RG	PR	25°26'20.44"S 53° 9'10.23"O	Stream	Stream, with the presence of forest in an advanced succession stage, inside the protected area.
8	2	RG	PR	25°26'19.53"S 53°10'11.56"O	Pond	Artificial pond, with the presence of pasture and agriculture in the surroundings, outside the protected area.
9	2	RG	PR	25°26'16.72"S 53°10'0.94"O	Stream	Stream, with the presence of riparian forest with secondary vegetation, outside the protected area.
10	3	RE	SC	26°22'3.39"S 52°50'28.57"O	Pond	Artificial pond with the presence of pasture, grass, and aquatic plants, inside the private reserve.
11	3	RE	SC	26°21'57.65"S 52°50'1.40"O	Stream	Second-order stream, with the presence of forest in an advanced succession stage, inside the private reserve.
12	3	RE	SC	26°22'2.64"S 52°49'47.29"O	Stream	Artificial pond, with the presence of pasture, close to urban area, outside the private reserve.
13	3	RE	SC	26°22'0.88"S 52°49'40.87"O	Stream	Stream spring, with the presence of pasture, grasses, and shrubs, close to urban area, outside the private reserve.
14	4	PA	SC	26°27'27.94"S 52°33'47.77"O	Pond	Pond with the presence of aquatic vegetation, grasses, and secondary forest in the surroundings, inside the protected area.
15	4	PA	SC	26°27'28.93"S 52°33'45.54"O	Stream	Stream, with the presence of secondary forest, inside the protected area.
16	4	PA	SC	26°28'8.27"S 52°34'17.88"O	Stream	Stream, with the presence of forest in an advanced succession stage, inside the protected area.

17	4	PA	SC	26°29'1.15"S 52°33'18.43"O	Pond	Pond, with the presence of aquatic vegetation, grasses, and secondary forest in the surroundings, pasture, and crops, outside the protected area.
19	5	QQ	SC	26°39'7.80"S 52°32'30.37"O	Pond	Artificial pond, with the presence of aquatic vegetation, grasses, pastures in secondary forest and lake, outside the private reserve.
20	5	QQ	SC	26°38'59.58"S 52°32'20.11"O	Stream	Stream, in secondary forest, close to pasture and lake, inside the private reserve.
21	6	FP	SC	27°17'29.56"S 52° 6'42.63"O	Stream	Stream, with the presence of forest in an advanced succession stage, inside the protected area.
22	6	FP	SC	27°17'21.45"S 52° 6'5.13"O	Pond	Artificial pond with the presence of aquatic vegetation, grasses, and secondary forest in the surroundings, pasture, and crops, outside the protected area.
23	6	FP	SC	27°17'37.40"S 52° 6'15.14"O	Stream	Stream spring with the presence of grasses and shrubs, outside the protected area.
24	6	FP	SC	27°17'30.69"S 52° 5'32.35"O	Stream	Stream with the presence of grasses and secondary riparian forest, outside protected area.
25	7	PT	RS	27°13'46.71"S 53°51'1.07"O	Pond	Pond with aquatic vegetation surrounded by secondary forest and close to road, inside protected area.
26	7	PT	RS	27°13'28.07"S 53°51'6.13"O	Pond	Pond with aquatic vegetation surrounded by secondary forest and close to road, inside protected area.
27	7	PT	RS	27°13'58.52"S 53°51'17.21"O	Stream	Lotic water, on the edge of secondary forest, inside protected area.

28	7	PT	RS	27°15'4.95"S 53°56'20.40"O	Pond	Semi-temporary artificial pond, surrounded by grassy vegetation. Outside protected area, in agriculture.
29	7	PT	RS	27°14'30.90"S 53°50'24.61"O	Pond	Semi-temporary artificial pond, surrounded by grassy vegetation. Outside protected area, in agriculture.
30	7	PT	RS	27°14'15.39"S 53°50'49.56"O	Stream	Lotic water, on the edge of secondary forest, near protected area, in agriculture.

Table S2. Average area (km²) of different categories of land use surrounding the sampled waterbodies estimated from a 500-m-radius buffer, in Atlantic Forest habitats in southern Brazil, from October 2018 to March 2019.

Waterbody	**Land use area (km²)**					
	Canopy cover	**Agriculture**	**Aquatic environment**	**Forest**	**Livestock farming**	**Urban area**
1	44.7	0.00	0.00	0.73	0.03	0.03
3	14.8	0.00	0.00	0.22	0.00	0.02
4	8.80	0.00	0.00	0.35	0.00	0.00
5	1.30	0.00	0.00	0.26	0.00	0.00
7	75.19	0.00	0.00	0.79	0.00	0.00
8	8.89	0.07	0.00	0.00	0.71	0.00
9	69.6	0.00	0.00	0.17	0.57	0.04
10	70.0	0.00	0.00	0.22	0.54	0.02
11	60.0	0.00	0.00	0.55	0.22	0.01
12	0.00	0.00	0.00	0.42	0.25	0.11
13	7.60	0.00	0.00	0.37	0.31	0.11
14	50.6	0.11	0.00	0.67	0.00	0.00
15	70.8	0.14	0.00	0.64	0.00	0.00
16	69.6	0.00	0.00	0.68	0.10	0.00
17	64.2	0.11	0.00	0.23	0.44	0.00
19	78.1	0.00	0.17	0.05	0.57	0.00
20	0.00	0.00	0.26	0.03	0.49	0.00
21	51.8	0.00	0.00	0.79	0.00	0.00
22	68.5	0.49	0.00	0.29	0.00	0.00
23	47.5	0.32	0.00	0.47	0.00	0.00
24	68.5	0.00	0.00	0.78	0.00	0.00
26	48.7	0.00	0.00	0.79	0.00	0.00
27	48.4	0.03	0.00	0.35	0.41	0.00
28	0.00	0.48	0.00	0.00	0.31	0.00
29	0.00	0.36	0.00	0.00	0.42	0.00
30	72.3	0.37	0.00	0.00	0.42	0.00

Table S3. Result of the Linear Mixed-Effect Models (LMM) showing the relationships between *Bd* prevalence and 500-m-buffer landscape variables in 26 waterbodies (breeding sites) of Atlantic Forest habitats in southern Brazil, recorded from October 2018 to March 2019. Num df = degrees of freedom numerator; Den df = degrees of freedom denominator.

Variable	**Num df**	**Den df**	***F***	***P-value***
Canopy cover	1	19	0.06	0.81
Forest	1	19	0.98	0.33
Agriculture	1	19	0.45	0.51
Livestock farming	1	19	0.22	0.64
Urban area	1	19	0.16	0.70
Aquatic environment	1	19	1.11	0.31

CONSIDERAÇÕES FINAIS

Este estudo representa um dos poucos trabalhos realizados com comunidades de girinos na região sul do Brasil, em escala de paisagem e abrangendo uma região relativamente extensa de Mata Atlântica de interior. Nesta região, grande parte da vegetação natural foi substituída por atividades de agropecuária, e existem poucos registros históricos relacionados à composição de espécies de anfíbios. Neste sentido, e por se tratar de um bioma com elevada diversidade e endemismos, estudos sobre diversidade e composição são fundamentais para direcionar estratégias de manejo e conservação da biodiversidade.

Nossos principais resultados adicionam conhecimento sobre composição e ocorrência das comunidades de girinos, os atributos de diversidade funcional dessas comunidades e a incidência de patógenos (*Batrachochytrium dendrobatidis* e ranavirus) relacionados a doenças preocupantes que já ocasionaram declínios populacionais de anfíbios em todo o mundo e estão presentes na Mata Atlântica. Nós utilizamos os atributos taxonômicos, ecomorfológicos e fisiológicos dos girinos como variáveis respostas à preditores ambientais, as características locais e de paisagem (uso de solo) e físico-químicas da água, como preditores explicativos. Nós registramos 22 espécies, sendo representadas majoritariamente pela família Hylidae e predominantemente por espécies generalistas de habitat, duas espécies especialistas de habitat (*Boana curupi* e *Crossodactylus schmidti*) e uma espécie exótica invasora (*Lithobates catesbeianus*).

As cinco principais classes de usos de solo na região de estudo foram: agricultura, ambientes aquáticos, florestas nativas, pastagens e urbanização. As classes de uso do solo predominantes foram florestas e pastagens, as quais foram responsáveis pela dissimilaridade na composição de espécies entre os corpos d’água. A riqueza de espécies não foi influenciada pelas classes de uso do solo.

Nosso estudo também apontou que os descritores locais do habitat foram mais importantes do que os descritores de paisagem na seleção dos atributos de diversidade funcional dos girinos, nos diferentes tipos de corpos d’água (riacho e lagoas). Observamos diferenças entre os tipos de corpos d’água, sendo os atributos de diversidade funcional dos girinos de riachos mais associados às características físico-químicas da água, presença de árvores nas margens e composição do substrato. Nas lagoas, a associação esteve mais relacionada com a profundidade, temperatura e pH da água. A partir dos dados em que analisamos a composição das comunidades e os atributos de diversidade funcional, podemos considerar que as características morfológicas do habitat (vegetação, solo, matéria orgânica e substrato) e físico-química da água são descritores importantes para a ocorrência e formação das comunidades de girinos nos ambientes aquáticos. Portanto, futuros estudos avaliando a diversidade filogenética também trariam resultados interessantes sobre a diversidade e ecologia dos girinos.

Além disso, nossos dados sobre a presença dos patógenos *Batrachochytrium dendrobatidis* (*Bd*) e o ranavirus, demonstraram ausência do ranavirus, mas elevada prevalência do fungo *Bd* nas comunidades de girinos estudadas. Mostramos também que as maiores áreas de florestas, cobertura vegetal original da região, e girinos com maior dependência destes ambientes, foram justamente os indicadores de *Bd*. Estes resultados são importantes pois, além de fornecer dados atualizados sobre a incidência de patógenos em comunidades de anuros ambientes naturais na região sul da Mata Atlântica, trouxe informações inéditas sobre o estado de infecção em girinos, demonstrando também que uso de técnicas histológicas e microscópicas podem ser adotadas para o diagnóstico do fungo.

Por fim, este estudo trouxe uma contribuição importante para o conhecimento sobre os girinos da região estudada. Contudo, é importante e necessário dar continuidade aos estudos para melhor compreender como a paisagem nos padrões de diversidade e incidência de doenças.