

Tiago Gomes dos Santos

## **Diversidade de anuros (Amphibia) do Parque Estadual Morro do Diabo, SP**

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro da Universidade Estadual Paulista "Júlio Mesquita Filho", como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Área de Concentração: Zoologia).

Rio Claro

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Orientador: Célio F.B. Haddad

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Comissão Examinadora

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Rio Claro, \_\_\_\_\_ de \_\_\_\_\_ de \_\_\_\_\_

## AGRADECIMENTOS

Sinto muito orgulho por ter contado com a ajuda de tantas pessoas para a realização desse trabalho. Ao mesmo tempo esta seção é motivo de tensão para mim, pois não gostaria de esquecer nome algum aqui. Bem, vamos lá.

Ao meu orientador, o Professor Célio Haddad, pela proposta inicial desse trabalho, pelos ensinamentos e pela confiança. A tua sobriedade e praticidade fizeram toda a diferença durante os períodos mais tensos desses quatro anos de Doutorado (tu sabes bem quais foram). Muito obrigado!

À Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), pelo apoio financeiro (processos nº 01/13341-3 e 02/13602-4) e ao Programa de Pós Graduação em Ciências Biológicas (Zoologia) pela bolsa Capes concedida.

À Direção do Parque Estadual do Morro do Diabo (PEMD), através do suporte logístico e constante incentivo que recebi da Andréa Soares Pires e do Helder Henrique de Faria. Trabalhar no PEMD, entre tantos bichos interessantes (não só da herpetofauna), foi incrível.

A todos os funcionários e estagiários do PEMD, pela amizade e solicitude. Em especial, agradeço àqueles que participaram de forma mais direta no projeto: Dona Ilda, Alex Alves (Duma), Alex R. Quilice, Eurico, Frans Artur, Gessi, Homero e Zé Gomes.

Às Dras. Cinthia Brasileiro (UNESP), Denise Rossa-Feres (UNESP), Marianna Dixo (USP) e Sonia Cechin (UFSM), pelas dicas no delineamento da amostragem com *pitfalls*.

Ao Dr. Hussam Zaher (MZUSP), pelas oportunidades e por todo o apoio logístico. Espero que em breve nosso artigo dos répteis do PEMD se torne realidade!

Ao Dr. João B. Baitello e à Dra. Giselda Durigan (Instituto Florestal), pelas dicas sobre a vegetação do PEMD.

Ao Dr. Raymond N. Gorley e ao Dr. K. Robert Clarke (Plymouth Marine Laboratory) pelas discussões acerca do SIMPROF e da PERMANOVA, respectivamente, no ambiente do PRIMER-E.

Aos intrépidos ajudantes de campo, que não mediram esforços nas longas horas das mais prazerosas atividades (e.g., retirar água com roedores das *pitfalls*, reparar centenas de metros de cercas-guia, cortar árvores caídas nas estradas, abrir

trilhas na mata, etc.): Carolina Panin Candeira, Fábio de Faria e Souza Campos, Fernando Rodrigues Silva, Janaína Rosa Cortinóz, Luciano Okayama, Luis Fernando Storti, Luis Gustavo Gorgatto da Silveira, Luiz Fernando Fracassi Gelin, Marcia Regina Spies, Rita Luiza Peruquetti, Thiago Telatim Tognolo, Thiago Motta de Oliveira, Tiago da Silveira Vasconcelos e Vitor Hugo Mendonça do Prado. Certamente as histórias com vocês renderiam outra Tese!

Ao Tiago da Silveira Vasconcelos, companheiro de projetos no Morro Diabo. Tê, não foi dessa vez que descremos o *Scinax diabolicus* nem o *Leptodactylus satanicus*, mas as experiências no Parque são para toda a vida! Sei que por vezes o Morro pareceu realmente ser 'do Diabo', mas no final das contas (após centenas de carrapatos, alguns sustos com vespas, onças, antas e caixacas) nem pegamos leishmaniose!!! Talvez a melhor designação agora seja Morro dos Tiago's!

Ao Luciano Okayama, pela amizade e por toda a ajuda no árduo início do projeto, com tantos imprevistos e tantas linhas de armadilhas para instalar.

Ao Fernando R. Carvalho e ao Luis Gustavo Gorgatto pela identificação dos peixes, à Marcia R. Spies pela ajuda na identificação dos insetos aquáticos e à Luciane Ayres-Peres pela ajuda com os crustáceos.

À Katia Kopp, pela amizade, pelas longas discussões estatísticas, pelas trocas de informações e de anseios.

A todos os amigos do Laboratório de Herpetologia pelo excelente convívio. Agradeço em especial à Cynthia Prado, Felipe Toledo, João Alexandrino, João Giovanelli, Juliana Zina, Luis Giasson, Maria Teresa Thomé, Olívia Araújo, Ricardo Ribeiro e Victor Dill pelos papos. Adicionalmente, agradeço ao Luis Giasson e ao Victor Dill pela hospedagem durante as disciplinas.

Aos amigos que fizeram o tempo passar mais rápido em Ribeirão Preto, Ana Emília Sieglöch, Karina Righi e Marcel Cavallaro.

Aos meus pais Antônio Jorge e Maria da Graça, meus pampeanos que ao longo dos anos têm se mostrado exímios coletores da herpetofauna! A distância é grande, mas o amor é maior, tenham certeza! Vocês me ensinaram a NUNCA desistir.

Ao meu irmão Lucas, pelo incentivo e pela torcida.

À Marcia Regina Spies, minha prenda que tem suportado ao longo dos últimos sete anos vários períodos de ausência (física e/ou mental), de estresse, de

ansiedade e de muita luta. Agradeço imensamente pelo teu amor, pelo apoio constante e pelo companheirismo. Te amo muito.

Como um bom ateu, eu não agradecerei a Deus, mas sim às fantásticas providências do acaso.

## Epígrafe

“Não tenho medo das chuvas tempestivas nem das grandes ventanias  
soltas, pois eu também sou o escuro da noite.”

**Clarice Lispector**

A Hora da Estrela

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## RESUMO

Estudamos a riqueza, composição taxonômica e os padrões de distribuição espaciais e temporais de taxocenoses de anuros do Parque Estadual Morro do Diabo (PEMD), o maior remanescente de Floresta Estacional Semidecídua no estado de São Paulo, Brasil. Registramos 28 espécies de anuros (Apêndice I) de setembro de 2005 a março de 2007, que compreenderam um misto de espécies de Mata Atlântica, do Cerrado e de formas amplamente distribuídas na América do Sul, geralmente consideradas tolerantes a modificações antropogênicas. A baixa riqueza de espécies e de modos reprodutivos, a predominância de espécies habitat-generalistas e a alta similaridade de espécies de anuros com áreas de Cerrado podem ser explicadas pela sazonalidade climática da área estudada (estação seca pronunciada), além da grande distância em relação a centros de diversificação de anuros, como as montanhas costeiras da Floresta Atlântica úmida. Chuva e fotoperíodo explicaram aproximadamente 77% da atividade de vocalização de toda a taxocenose, enquanto somente a chuva e o fotoperíodo explicaram a temporada de vocalização em ambientes temporários e permanentes, respectivamente. Registramos alta sobreposição na temporada de vocalização dos machos, mas segregação na fase larval. A distribuição das espécies de anuros entre sítios de reprodução (Apêndice II) diferiu da esperada pelo acaso e compreendeu três taxocenoses distintas de anuros que foram explicadas pelo conjunto de variáveis ambientais de riachos permanentes, represas permanentes e poças temporárias. Registramos que 19 espécies de anuros (aproximadamente 83% da riqueza total de espécies registradas nos corpos d'água monitorados) foram indicadoras da heterogeneidade ambiental: três espécies indicaram riachos permanentes, quatro indicaram represas permanentes e 12 espécies indicaram poças temporárias. Em relação à distribuição micro espacial das espécies em duas poças temporárias do PEMD, registramos que machos da maioria dos pares de espécies testados (96%) usaram sítios distintos para as atividades de vocalização. A combinação de variáveis que melhor discriminou as espécies de anuros em relação aos sítios de vocalização dos machos foi composta por tipo de substrato, micro relevo das poças e tipo de poça. Além disso, o tipo de substrato, micro relevo das poças, profundidade da água e altura dos poleiros isoladamente também apresentaram força discriminatória. Os

padrões de similaridade no uso dos sítios de vocalização não tiveram relação com a distância filogenética entre as espécies de anuros. Assim, tais padrões podem ser explicados principalmente pela heterogeneidade ambiental das poças, bem como pela plasticidade ecológica das espécies.

**Palavras-chave:** Taxocenoses de anuros. Domínio Atlântico. Análise de similaridade. Teste de hipóteses. Padrões temporais. Distribuição espacial.

## **ABSTRACT**

We studied richness, composition, and patterns of temporal and spatial distributions of anuran assemblages of Morro do Diabo State Park (MDSP), the major remnant of Mesophytic Semideciduous Forest (MSF) in the state of São Paulo, Brazil. From September 2005 to March 2007 we recorded 28 anuran species (Appendix I), comprising a mix of Atlantic, Cerrado, and South American widespread species, usually considered tolerant to anthropic modifications. The low richness of species and reproductive modes, the predominance of habitat generalist species, and the high similarity with Cerrado areas can be explained by climatic seasonality of the studied area (pronounced dry season), besides its large distance in relation to centers of anuran diversification, such as coastal mountains of the wet Atlantic Forest. Rainfall and photoperiod explained about 77% of calling activity of the whole assemblage, while rainfall alone in temporary habitats and photoperiod in permanent ones explained the calling season. We recorded high temporal overlap for calling males, but segregation for tadpoles. Spatial distribution of anuran species among breeding sites of the MDSP (Appendix II) differed of expected by chance and comprised three distinct anuran assemblages that were explained by the suite environmental variables of permanent streams, permanent dams, and temporary ponds. We recorded that 19 species (about 83% of total anuran species recorded in monitored sites) were indicators of environmental heterogeneity: three anuran species indicated permanent streams, four indicated permanent dams, and 12 anuran species indicated temporary ponds. Regarding to micro-spatial distribution of anuran species at two temporary ponds of MDSP, we recorded that males of most pairs of species (96%) used distinct sites for calling activities. The best combination of variables discriminating anuran species regarding male calling sites was type of substratum, pond micro-relief, and pond type. In addition, the type of substratum, pond micro-relief, water depth, and perching height alone also presented discriminatory power. The similarity patterns in calling site use have no relation with phylogenetic distance among anurans species. Thus, such patterns can be explained mainly by environmental heterogeneity of ponds, as well as by the ecological plasticity of anuran species.

**Keywords:** anuran assemblages, Atlantic Domain, similarity analysis, hypotheses tests, temporal patterns, spatial distribution.

## INTRODUÇÃO

Na região neotropical, onde ocorre a maior riqueza de espécies de anuros (Crump 1974, Heyer et al. 1990), estudos de comunidades ainda são escassos, apesar da valiosa informação que este tipo de trabalho pode fornecer (Duellman & Trueb 1994), como padrões de diversidade local (riqueza, abundância e equitabilidade) e de distribuição espaço-temporal das espécies (e.g., Cardoso et al. 1989, Aichinger 1992, Haddad & Sazima 1992, Rossa-Feres & Jim 1994, Pombal 1997).

No Brasil, mais de 800 espécies de anuros são registradas (SBH 2008), tornando o país o mais diverso no mundo em relação a este grupo. Entretanto, como os estudos foram historicamente desenvolvidos na região costeira e ao longo dos grandes rios (Haddad 1998), o conhecimento da biologia e ecologia da maioria das espécies é escasso, especialmente nas áreas interioranas. A destruição indiscriminada de habitats (e.g., desmatamento, expansão agrícola) é considerada a principal ameaça à conservação de anfíbios no Brasil (Silvano & Segalla 2005), mas a falta de dados básicos sobre taxonomia, distribuição geográfica e uso de habitats obstrui a avaliação do status de conservação de muitas espécies (Pimenta et al. 2005).

Com relação aos padrões de distribuição espacial, a maioria dos ecologistas assumiu que comunidades animais não são simplesmente grupos aleatórios de espécies (Wells 2007). Por isso, fatores determinísticos foram destacados como responsáveis pela variação da diversidade e da composição de espécies ao longo de gradientes ambientais e/ou espaço-temporais (Chase & Leibold 2003). Entretanto, de acordo com a recente Teoria da Neutralidade de Hubbell, a estrutura de taxocenoses resulta de processos estocásticos e não sofre influência de características das espécies ou de condições ambientais (ver detalhes em Tilman 2004 e Chase 2007).

Estudos recentes destacaram que taxocenoses neotropicais possuem estruturação fraca ou ausente (ver exemplo em Eterovick & Barros 2003 e Afonso & Eterovick 2007 para anuros, e em França & Araújo 2007 para serpentes), fortalecendo a Teoria da Neutralidade. Entretanto, muitos estudos também destacaram que habitats similares e próximos entre si apresentam pequenas

diferenças em relação à composição das taxocenoses de anfíbios, já que algumas espécies vivem em uma variedade de habitats e outras possuem requerimentos de habitat mais especializados (ver referências em Snodgrass et al. 2000 e Wells 2007). Assim, diferenças nas condições ambientais de um habitat para outro explicam as diferenças entre taxocenoses, como a riqueza e a abundância de espécies em taxocenoses de anfíbios (ver referências em Toft 1982).

Dentro de uma taxocenose, guildas de anuros usam sítios de vocalização de forma distinta, já que espécies cujos machos vocalizam empoleirados na vegetação parecem ter mais oportunidades para segregação micro-espacial que espécies cujos machos vocalizam no solo ou flutuando na superfície da água (Rossa-Feres & Jim 2001, Santos & Rossa-Feres 2007, Vasconcelos & Rossa-Feres 2008). Assim, a diferenciação interespecífica dentro de um corpo d'água parece representar preferências específicas dos anuros por determinados micro-habitats (Kopp & Eterovick 2006, Wells 2007).

Preferências por micro-habitats são consideradas adaptativas, potencialmente refletindo seleção natural sobre escolhas de habitat (Martin 1998). Assim, a escolha de sítios de vocalização pode estar relacionada aos modos de reprodução, bem como a limitações morfológicas, fisiológicas ou comportamentais das espécies (Crump 1971, Cardoso et al. 1989).

De fato, fatores históricos representam uma importante função na estruturação das taxocenoses contemporâneas (Cadle & Greene 1993). Assim, espécies filogeneticamente próximas podem apresentar alta similaridade ecológica, tal como registrado para lagartos (Poe 2005) e serpentes (França et al. 2008). Para anuros, é esperado que espécies relacionadas possuam alta similaridade ecológica, mas ocupem partes sutilmente distintas do habitat, resultando em segregação espacial (Heyer et al. 1990). Os poucos estudos que relacionaram padrões de similaridade no uso de sítios de vocalização com a proximidade taxonômica/filogenética de anuros registraram um efeito parcial ou fraco da proximidade das espécies em relação ao uso de micro-habitats (Rossa-Feres & Jim 2001, Eterovick et al. 2008, respectivamente).

De forma geral, a temporada de reprodução em anuros é principalmente relacionada a fatores abióticos locais, como a distribuição sazonal da chuva e a disponibilidade e hidroperíodo de ambientes de reprodução (Gottsberger & Gruber

2004, Wells 2007). Assim, a atividade reprodutiva em regiões temperadas úmidas é concentrada no período quente do ano, enquanto em regiões tropicais e subtropicais é relacionada ao período quente e chuvoso (Duellman & Trueb 1994, Wells 2007). Além disso, em ambientes tropicais não sazonais úmidos e quentes é esperado que ocorra atividade reprodutiva ao longo de todo ano (Crump 1974), o que permite oportunidades para segregação temporal entre espécies (Duellman 1978). Por outro lado, é esperado que um padrão reprodutivo concentrado predomine em ambientes tropicais sazonalmente secos, com alta sobreposição de espécies durante a estação chuvosa (e.g., Rossa-Feres & Jim 1994, Prado et al. 2005, Vasconcelos & Rossa-Feres 2005, Santos et al. 2007).

Estudos sobre taxocenoses de anfíbios em áreas subtropicais da América do Sul não têm registrado um único padrão de atividade reprodutiva, já que a maior parte da atividade dos anuros foi explicada somente pela temperatura (Bertoluci 1998, Bernarde & Machado 2001), pela chuva (Conte & Rossa-Feres 2007), pela chuva e temperatura (Conte & Machado 2005, Conte & Rossa-Feres 2006), ou ainda por nenhum desses fatores (Pombal 1997, Bernarde & Anjos 1999). Além disso, estudos de taxocenoses mais austrais têm revelado que a chuva, a umidade do ar ou a temperatura não foram preditores adequados da atividade de vocalização dos anuros (Both et al. 2008, Canavero et al. 2008, Santos et al. 2008), já que o fotoperíodo e um modelo sinusoidal representando a sazonalidade circanual latente explicaram a maior porção da atividade de vocalização das espécies (Both et al. 2008, Canavero et al. 2008, respectivamente).

Aqui nós apresentamos um estudo sobre os padrões de distribuição espacial e temporal da anurofauna do Parque Estadual Morro do Diabo (PEMD), o maior remanescente da Floresta Estacional Semidecídua do estado de São Paulo (Durigan & Franco, 2006). Em todo o país, este tipo de floresta foi quase totalmente devastado devido à alta fertilidade do solo, ao relevo suave, à alta disponibilidade de madeira de lei e a interesses políticos inescrupulosos (Murphy & Lugo 1986, Prado & Gibbs 1993, Dean 1998). Assim, este é o tipo de floresta tropical mais ameaçado do mundo (Jansen 1997), já que somente 2% de sua cobertura original ainda permanecem atualmente, de forma muito fragmentada (Viana & Tabanez 1996, Werneck & Colli 2006).

A Floresta Estacional Semidecídua também foi historicamente negligenciada quanto à criação de Unidades de Conservação, devido à baixa taxa de endemismos, se comparada com as florestas úmidas (Jansen 1997, Prado 2000, Pennington et al. 2006). Essa realidade pode explicar o limitado conhecimento existente sobre a herpetofauna dessa fisionomia.

Para lagartos, por exemplo, recentes estudos (Werneck & Colli 2006) confirmam a importância da Floresta Semidecídua como uma nova unidade fitogeográfica (conhecida como 'Região das Florestas Tropicais Sazonais' *sensu* Prado 2000), a qual necessita urgentemente de estudos e de iniciativas conservacionistas. Entretanto, generalizações acerca dos padrões de diversidade de anuros nessa fisionomia permaneciam especulativas. Até a última década, havia a expectativa de que a anurofauna da Floresta Estacional Semidecídua no sudeste do Brasil era menos diversa do que a conhecida para as florestas úmidas, mas possivelmente mais diversa do que a registrada no Cerrado (Haddad 1998). Entretanto, a falta de estudos sobre áreas preservadas de Floresta Estacional Semidecídua no estado de São Paulo limitou a avaliação de possíveis perdas ou substituições de espécies em áreas degradadas, originalmente recobertas por este tipo de vegetação (Santos et al. 2007). Então, análises recentes mostraram que a composição da anurofauna em remanescentes de Floresta Estacional Semidecídua no interior de São Paulo parece incluir espécies típicas da Floresta Atlântica úmida, do Cerrado, ou de áreas perturbadas (Bertoluci et al. 2007, Zina et al. 2007), mas o desenvolvimento de futuras pesquisas nesse tipo de floresta foi destacado como ainda necessário (Zina et al. 2007), justificando o presente estudo.

A presente Tese foi dividida em quatro capítulos, de acordo com quatro enfoques principais, como descritos abaixo:

- Capítulo 1. Este capítulo foi aceito para publicação no periódico *Journal of Natural History* e trata basicamente da riqueza e composição taxonômica da anurofauna registrada no PEMD. Desta forma, foram utilizadas análises de similaridade e testes de hipóteses para tentar estabelecer uma visão geral sobre a taxocenose estudada, considerando as afinidades com outras taxocenoses previamente registradas em diferentes fitofisionomias do Brasil.

- Capítulo 2. Este capítulo foi submetido à publicação no periódico *Annales Zoologici Fennici*, mas o resultado da avaliação ainda não foi divulgado. Aqui o

enfoque foi a análise dos padrões de distribuição temporal da anurofauna do PEMD. Assim, a influência de fatores climáticos sobre a temporada de vocalização das espécies foi testada em duas escalas, considerando toda a taxocenose do PEMD e apenas aquela registrada em seis corpos d'água mensalmente monitorados. Além disso, foi abordada a partilha temporal durante a fase adulta e larval das espécies.

- Capítulo 3. Este capítulo ainda não foi submetido à publicação. A ênfase nesse estudo foi a distribuição espacial das espécies de anuros em seis corpos d'água mensalmente monitorados no PEMD. Os padrões espaciais registrados para a anurofauna foram discutidos com base em características específicas dos corpos d'água e na ecologia das espécies. Além disso, foram destacadas possíveis implicações dos resultados obtidos para estratégias de conservação de anuros da Floresta Estacional Semidecídua.

- Capítulo 4. Este capítulo ainda não foi submetido à publicação. A abordagem aqui ficou por conta da distribuição micro-espacial dos machos durante a atividade de vocalização em duas poças temporárias do PEMD. Foram realizados testes para determinar se as espécies de anuros diferiram quanto ao uso dos sítios de vocalização, e se ocorreu grande variabilidade intraespecífica. Finalmente, foram determinadas as variáveis ambientais (ou conjuntos de variáveis) que melhor discriminaram as espécies de anuros em relação ao uso dos sítios de vocalização.

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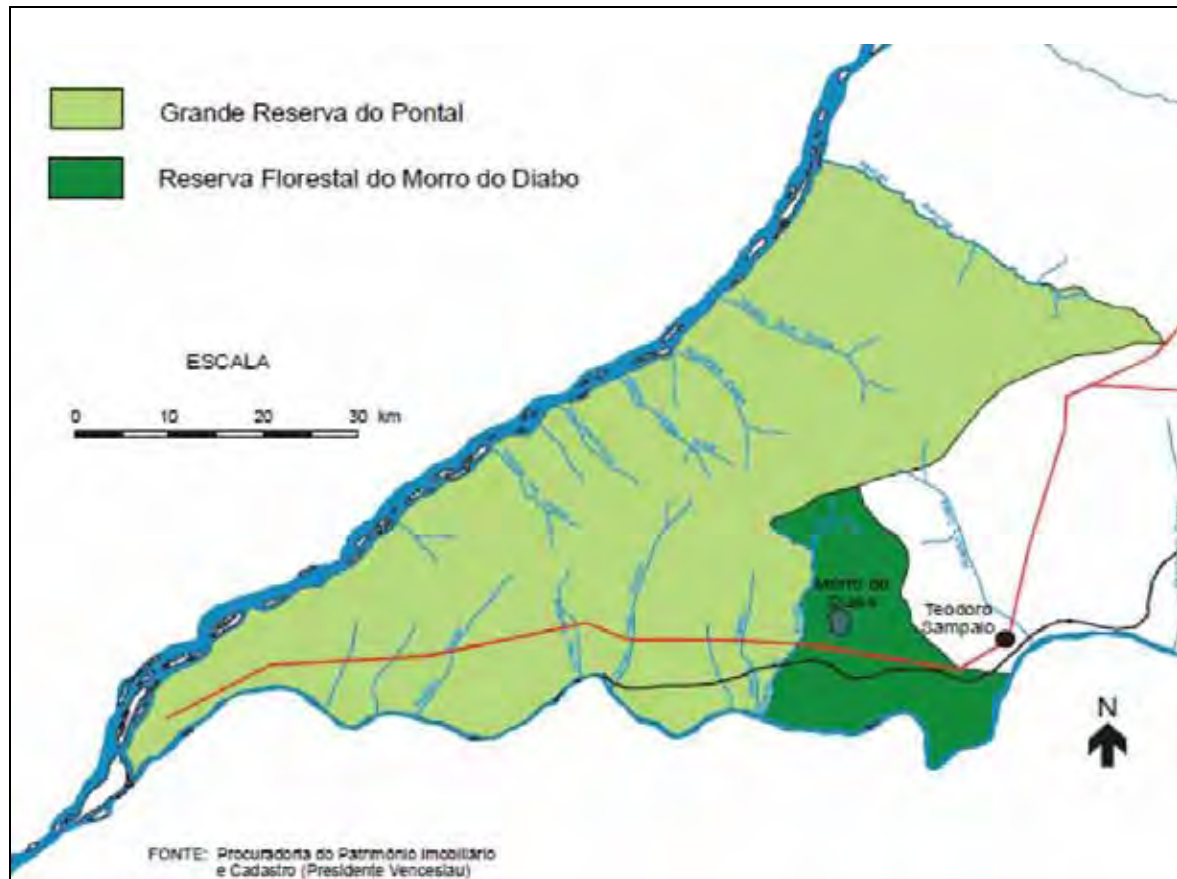
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# CAPÍTULO 1

ANURANS OF A SEASONALLY DRY TROPICAL FOREST: MORRO DO DIABO STATE PARK, SÃO  
PAULO STATE, BRAZIL

## **ANURANS OF A SEASONALLY DRY TROPICAL FOREST: MORRO DO DIABO STATE PARK, SÃO PAULO STATE, BRAZIL**

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### **Abstract**

We studied richness and composition of the anuran assemblage of Morro do Diabo State Park (MDSP), the major remnant of Mesophytic Semideciduous Forest (MSF) in the state of São Paulo, Brazil, through hypothesis tests. From September 2005 to March 2007 we recorded 28 anuran species, comprising a mix of Atlantic, Cerrado, and South American widespread species, usually considered tolerant to anthropic modifications. The low richness of species and reproductive modes, the predominance of habitat generalist species, and the high similarity with Cerrado areas can be explained by climatic seasonality of the studied area (pronounced dry season), besides its large distance in relation to centers of anuran diversification, such as coastal mountains of the wet Atlantic Forest.

**Keywords:** Anurans; composition, Atlantic Domain; similarity analysis; hypothesis tests

### **INTRODUCTION**

The historical process of habitat loss and fragmentation is possibly the most important environmental alteration caused by man (Cerqueira et al. 2003), since it

constitutes a very serious threat to the diversity of countless groups of animals and plants (Laurance and Bierregaard Jr. 1997; Lips 1999; Primack and Rodrigues 2001; Haddad 2005). Amphibians in particular, due to their complex life cycle, highly permeable skin, low mobility, and special physiological requirements, have their diversity and distribution negatively affected by environmental alterations (Beebee 1996; Tocher et al. 1997; Pough et al. 2001; Krishnamurthy 2003; Cushman 2006; Becker et al. 2007; Garder et al. 2007).

In Brazil, more than 800 species of anurans are recorded (SBH 2008), making the country the most diverse in the world regarding this group. However, since studies have historically been undertaken in coastal areas and along main rivers (Haddad 1998), knowledge of the biology and ecology of most species is scarce, especially in inland areas. The unrestrained habitat destruction (e.g. deforestation, agricultural expansion) is considered to be the main threat to Brazilian amphibian conservation (Silvano and Segalla 2005), but the lack of basic data about anuran taxonomy, geographical distribution, and habits, obstruct the evaluation of the conservation status of many species (Pimenta et al. 2005).

Despite having been more studied than the Cerrado, the Atlantic Forest Domain (*sensu* Ab'Saber 1977) still needs further medium and long-term studies and surveys on anurans (Haddad and Sazima 1992). In the Atlantic Forest Domain, the Semideciduous Forests (or Inland Atlantic Forest) were almost totally devastated due to their soil fertility, smooth relief, high availability of valuable hardwood, and because of unscrupulous political interests (Murphy and Lugo 1986; Prado and Gibbs 1993; Dean 1998). Besides, the Semideciduous Forests have been historically neglected as areas for conservation unit creation due to their low level of endemism, when compared to the humid forests (Jansen 1997; Prado 2000; Pennington et al. 2006). It may also explain the limited knowledge concerning its herpetofauna. However, recent studies of lizards (Werneck and Colli 2006) confirm the importance of the Semideciduous Forests as a new phytogeographic unit (known as 'Tropical Seasonal Forests Region' *sensu* Prado 2000), which need urgent studies and conservation initiatives. It is the most threatened type of tropical forest in the world (Jansen 1997), with only a very fragmented 2% of its original cover remaining (Viana and Tabanez 1996; Werneck and Colli 2006).

Until the last decade, anuran amphibians were expected to be less diverse in the Mesophytic Semideciduous Forest in southeast Brazil than in the humid rain forests, but more diverse when compared to the Cerrado (Haddad 1998). However, the lack of studies on pristine Mesophytic Semideciduous Forest areas in São Paulo state limits the evaluation of possible anuran species loss or substitution in degraded areas, originally covered by this type of vegetation (Santos et al. 2007). Recent analyses have shown that anuran species composition in Mesophytic Semideciduous Forest remnants in inland São Paulo state seem to include typical species from the Atlantic Rain Forest, the Cerrado, or disturbed areas (Bertoluci et al. 2007; Zina et al. 2007), but the development of further studies on this type of forest is still necessary (Zina et al. 2007).

The objectives of this study were: i) to determine the richness and the composition of the anuran assemblage of Morro do Diabo State Park (MDSP) – the largest remnant of Semideciduous Forest in São Paulo state; ii) to determine the MDSP anuran assemblage similarity with other assemblages in different phytogeographic units; iii) to test if the similarity in the composition of species among the analyzed locations is influenced by the type of phytogeographic unit and by the geographical distance.

## **MATERIAL AND METHODS**

### **Study site**

This study was carried out in Morro do Diabo State Park (MDSP), located in the municipality of Teodoro Sampaio in the extreme west of São Paulo state (22°27' to 22°40' S and 52°10' to 52°22' W; 260 to 599.5 m in altitude), in the Pontal do Paranapanema zone, which belongs to the hydrographic basin of the Paranapanema River. In the 1940's, more than 290,000 ha of native habitat in the Pontal do Paranapanema were protected by law, but soil exploitation greatly reduced vegetation cover, and today only 1.85% of the original area remains (Bensusan 2006), mainly represented by MDSP. Nowadays, the park is considered the major Mesophytic Semideciduous Forest (MSF) remnant area in São Paulo state (Projeto BRA/90/010 1995; PROBIO 1998) and one of the four largest (>10,000 ha) protected areas of MSF in the country, accounting for 33,845 ha (Durigan and Franco 2006) (Figure 1). The area was indicated as a priority for amphibian and reptile surveys in

São Paulo state (Haddad and Abe 2000) and it is considered as “insufficiently known, but with probable biological importance” in relation to the diversity of these groups (Haddad 2002; Rossa-Feres et al. in press).

The MSF is the predominant vegetation type in the park, but the area is characterized as a mosaic of forests in different stages of regeneration, and a small area of Cerrado *sensu stricto*, surrounded by a transition forest (Myrtaceae forest) (Durigan and Franco 2006). The MSF is considered one of the subtypes of the Atlantic Forest Domain (*sensu* Ab' Saber 1977). It is characterized by the absence of conifers and by the partial loss of leaves, as a consequence of low rainfall during the winter (Veloso et al. 1991). The MSF is also interpreted as a remnant of the Pleistocenic Arc, which widely extended itself over South America during a cooler and dryer period that is coincident with the retraction of humid forests (Prado and Gibbs 1993; Pennington et al. 2000). The discussions concerning this type of forest identity are still open since it is considered as a new phytogeographic unit (Prado and Gibbs 1993; Prado 2000), or as presenting reasonable affinity with the humid Atlantic Forest (Oliveira-Filho and Fontes 2000; see revision in Farley 2007).

The weather in this region is subtropical, characterized by dry winters and hot and wet summers (Cwa of Köppen). The average annual rainfall varies from 1,100 mm to 1,300 mm and the humid season extends from September to March (Figure 2). The average annual temperature in the region is 22°C and the minimum and maximum extreme averages are 10°C and 35°C, respectively. Frosts may occur during the coldest period of the year (Faria 2006). The relative humidity is high (averaging 80%), even during the driest period of the year (Faria 2006).

### **Sampling methods**

Complementary sampling techniques were used in order to carry out this MDSP anuran survey: i) pitfall traps with drift fences (Corn 1994; Cechin and Martins 2000), ii) surveys at breeding sites (*sensu* Scott Jr and Woodward 1994), and iii) tadpole sampling.

Pitfall traps with drift fences were installed in five MDSP vegetation types: climax forest, initial regeneration of Mesophytic Semideciduous Forest, advanced regeneration of Mesophytic Semideciduous Forest, Myrtaceae Forest, and Cerrado *sensu stricto*. Six sets of traps were installed in the climax Mesophytic

Semideciduous Forest, the vegetation type with the largest coverage area in MSDP. For all other vegetation types, three sets of traps were installed. Each set was composed of ten 100 liter plastic buckets arranged in line every 10 m and linked by a 90 cm high plastic canvas. The traps remained open for six consecutive days in every month, accounting for 1,728 sampling hours/bucket from October 2005 to September 2006. The inspection of the traps was made every 48 hours, always in the morning, with a total of three inspections per month. Captured individuals were identified, marked by clipping one toe (adapted from Martof 1953), and immediately released at the site of capture. The toe clipping marking technique was applied in order to study the spatial distribution of anurans among different types of vegetation inside MDSP (Santos and Vasconcelos, unpubl. data).

Surveys at breeding sites were conducted in 12 water bodies (two permanent streams, three permanent dams, three permanent and four temporary ponds), which were monitored monthly from November 2005 to March 2007. The search for anurans was made along the perimeters of breeding sites and 500 m sections of stream banks using visual encounters and also listening for males engaged in calling activities. The sampling effort in monitored breeding sites varied according to size and complexity (*sensu* Scott and Woodward 1994) and was shorter during the dry season, when a few anurans species were recorded in MDSP (T.G. Santos unpubl. data).

Tadpole sampling was carried out in the same breeding sites where the adults were monitored. The collection was made monthly (from November 2005 to March 2007), with a long wire hand net (3 mm<sup>2</sup> mesh size). The effort was standardized by passing the net along the banks of ponds and streams, intending to sample all the available microhabitats. The collected tadpoles were fixed in formalin 10% and identified in the laboratory following Cei (1980) and Rossa-Feres and Nomura (2006).

Occasional records of adults and tadpoles in other breeding habitats in MDSP and adjacent areas (e.g. Paranapanema River banks, swamp areas, ephemeral ponds, artificial dams, rain formed temporary streams and ponds) were also considered. Voucher specimens were deposited in the anuran collections CFBH (Célio F.B. Haddad, UNESP - Rio Claro, SP, Brazil) and DZSJRP (Departamento de Zoologia e Botânica, UNESP – São José do Rio Preto, SP, Brazil).

## Statistical Analysis

The evaluation of collection effectiveness was undertaken by a species accumulation curve (collector curve) and by five quality estimators (Bootstrap, Chao II, ICE, Jackknife I e II; see references in Santos 2003), based only in records of surveys at breeding sites. The records of pitfall traps with drift fences and tadpole sampling were not used for statistical estimations because they produced only subsets of the species richness recorded by the surveys at the breeding sites.

The recorded MDSP anuran composition was compared to the species lists from other locations, in different phytogeographic units in the country: Caatinga, Cerrado, Atlantic Forest *sensu stricto*, Mesophytic Semideciduous Forest, Amazonian Forest, Pampa and Pantanal (Table 1). According to Pombal (1995) and Bastos et al. (2003), comparisons of species lists can be affected by differences in sampling effort, taxonomic concepts, size of sampled area, characteristics, and conservation status of the locations. As highlighted by Bastos et al. (2003), the low availability of Brazilian locations with faunistic inventories prevents the simple change of one checklist by another more comparable in such analyses. In the present study, the comparison of species lists in the similarity analysis could be affected by the limitations mentioned above, since the studies varied widely in sampling effort and schedules (Table 1). However, this bias was minimized because studies with a low sampling effort in a temporal axis had a high sampling effort in a spatial axis (e.g., Haddad et al. 1988, Strüssmann 2000, Uetanabaro et al. 2007). On the other hand, some species lists included in the similarity analysis, which did not have accurate information about sampling effort and schedule employed (e.g., Heyer et al. 1990, Machado et al. 1999, Izecksohn and Carvalho-e-Silva 2001a, 2001b), summarized the results of several years of study and for this reason, they are representative of the sampled anuran assemblages.

The similarity among communities was calculated using the Coefficient of Geographic Resemblance (CGR) (Duellman 1990):  $CGR = 2N_S / N_A + N_B$ , where  $N_S$  represents the number of species in both areas;  $N_A$  represents the number of species in area A and,  $N_B$  represents the number of species in area B. The index varies from 0 (maximum dissimilarity) to 1 (maximum similarity). In order to compose the data matrix (list of species), only the *taxa* listed at a specific level in the literature were considered.

The similarity matrix (CGR) was represented in a later weighted pair-group method with an arithmetic average (WPGMA) (Sokal and Michener 1958), in order to avoid the effect of sample size (species richness in different communities) on the analysis (Valentin 1995). Possible distortions in the graphic representation of the similarity matrix caused by the pair group method used were evaluated by the Cophenetic Correlation Coefficient ( $r$ ) (Romesburg 1984). The coefficient is obtained by correlating the original similarity matrix with the obtained matrix from the dendrogram, where  $r \geq 0.9$  is considered a very good fit;  $0.8 \leq r < 0.9$  is considered a good fit,  $0.7 \leq r < 0.8$  is considered a poor fit, and  $r < 0.7$  is considered a very poor fit (Rohlf 2000).

The influence of the type of phytogeographic unit and of the geographical distance on the similarity matrix concerning the faunistic compositions among the studied locations (CGR) was determined by Mantel's test (Manly 2000). This test performs the matrix correlations, using the  $Z$  statistic, where  $Z$  depends on the number and magnitude of elements in the matrix to be compared. Consequently, a normalization is performed to transform  $Z$  in one coefficient ( $r$ ) that varies from +1 to -1. The  $Z$  significance was determined by a Monte Carlo permutation test (Smouse et al. 1986), using 5,000 permutations.

For Mantel's test, four matrices were built, based on four hypothesis, to be compared to the similarity matrix of faunistic composition:

- i) A similarity matrix (MS1) considering six phytogeographic units (Caatinga, Cerrado, Amazonian Forest, Atlantic Forest, Pampa and Pantanal), including the locations situated in the extension of original Mesophytic Semideciduous Forest as part of the Atlantic unit (*sensu* Projeto BRA/90/010 1995; PROBIO 1998; Oliveira-Filho et al. 2006).
- ii) A similarity matrix (MS2) considering six phytogeographic units (Caatinga, Cerrado, Amazonian Forest, Atlantic Forest, Pampa and Pantanal), plus the locations situated in the extension of original Mesophytic Semideciduous Forest as a seventh phytogeographic unit (*sensu* Prado 2000).
- iii) A similarity matrix (MS3) considering six phytogeographic units (Caatinga, Cerrado, Amazonian Forest, Atlantic Forest, Pampa and Pantanal),

including the areas of Mesophytic Semideciduous Forest as part of the Cerrado unit.

- iv) A dissimilarity matrix (MDG) considering the geographical distance between the locations.

In order to have the similarity matrix, pairs of locations that belong to different phytogeographic units received value 0, and pairs of locations that belong to the same phytogeographic unit received value 1. The geographical distance (in km) between the locations was measured using GPS TrackMaker 13.0<sup>®</sup> software.

When a significant influence regarding the geographical distance was verified on the CGR matrix, new correlations were made with the hypothesis matrix, using the Partial Mantel test (Smouse et al. 1986), to take out the distance effect among the locations on the other correlations' results. The method consists of comparing two matrices (A and B), removing the effect of a third matrix (C) on them, using a regression of C on A and B, obtaining a residues matrix that represent the variation of the matrices A and B, which is not explained by matrix C (Smouse et al. 1986). In this way, the two residue matrices can be compared normally. The statistical analyses were performed using NTSYSpc 2.10s software (Rohlf 2000).

## RESULTS

### Species richness and taxonomic composition

In 19 field expeditions, we recorded 28 species of anurans in MDSP and surroundings, distributed between six families: Bufonidae (02), Cycloramphidae (01), Hylidae (14), Leiuperidae (02), Leptodactylidae (07) and Microhylidae (02) (Table 2).

All the 28 anuran species listed for MDSP were recorded during the sampling in breeding sites, 20 species were also recorded by tadpole sampling and only seven by pitfall traps (Table 2). The species accumulation curve analysis, based on the sampling at breeding sites, produced a clear asymptotic shape and little variation associated to the mean curve from the 12th sample (Figure 3). The richness estimators produced stable estimations, close to the observed richness (from  $26.31 \pm 0$  to  $28.32 \pm 0$  species) (Figure 3).

The anurans of MDSP exhibited a total of seven reproductive modes (*sensu* Haddad and Prado 2005) (Table 2). The deposition of eggs in ponds, with exotrophic tadpoles developing in water (mode 1) was the most frequent reproductive mode

observed among the 28 species recorded (n = 15 species; 52%). The deposition of eggs in foam nests on the water surface with exotrophic tadpole development in ponds (mode 11) was the second most frequent reproductive mode in the studied area (n = 4 species; 14%); followed by the deposition of eggs in streams with exotrophic tadpoles developing in streams (mode 2), and the deposition of eggs in foam nests in subterranean burrows with exotrophic tadpoles developing in ponds (mode 30) (both with n = 3 species; 9% each). The deposition of eggs in foam nests on the surface of water accumulated in a constructed basin, with exotrophic tadpoles developing in ponds (mode 13) was not frequent in the area, neither were the deposition of eggs on the water surface in natural or constructed basins, with exotrophic tadpoles developing in ponds or streams (mode 4) (both with n = 2 species; 7% each), and the deposition of eggs in foam nests in subterranean burrows with exotrophic tadpoles development in streams (mode 31; n = 1 species or 2%). Two species (*Hypsiboas albopunctatus* and *Leptodactylus mystaceus*) presented behavioral plasticity and used more than one reproductive mode (Table 2).

### Comparison with other locations

The MDSP anuran similarity analysis with other studied areas in different phytogeographic units in the country indicated the existence of two groups with a similarity higher than 25% (Figure 4): 1) one represented by assemblages that belong to a mosaic of the Mesophytic Semideciduous Forest (including those nowadays converted into agricultural and pasture areas), Cerrado, Pampa and Pantanal; and 2) one represented by the assemblages of Atlantic Forest *sensu stricto* and the Mesophytic Semideciduous Forest of Serra do Japi (FES6). The Amazonian Forest and the Caatinga presented low similarity to other areas (< 25%), although they have demonstrated some relation to the group 1 (Figure 4).

The Mantel's test showed that the faunistic similarity among the studied areas (CGR) was positively correlated with all the hypothesis matrices tested, considering the types of phytogeographic units (MS1, MS2 and MS3) (Table 3). However, the similarity among the areas was negatively correlated with the geographical distance (MDG) between them (Figure 5; Table 3). Accordingly, after excluding the geographical distance effect, a greater congruence between the faunistic similarity and the hypothesis matrix of the phytogeographic unit MS3

became evident (considering the Mesophytic Semideciduous Forest as a Cerrado unit), followed by the hypothesis matrix MS2 (considering the Mesophytic Semideciduous Forest as a new phytogeographic unit) (Table 3). The faunistic similarity matrix was not correlated with the hypothesis matrix MS1, in which the Mesophytic Semideciduous Forest is part of the Atlantic phytogeographic unit (Table 3).

## DISCUSSION

Thirteen (46%) of the 28 recorded species in this study consist of new records for MDSP (Dixo et al. 2006): *Chiasmocleis albopunctata*, *Dendropsophus minutus*, *Elachistocleis bicolor*, *Eupemphix nattereri*, *Hypsiboas faber*, *H. punctatus*, *Itapotihyla langsdorffii*, *Leptodactylus chaquensis*, *L. labyrinthicus*, *L. mystaceus*, *L. mystacinus*, *Odontophrynus americanus* and *Scinax* cf. *similis*. Among them, the recording of *Hypsiboas punctatus* was the first in southeast Brazil (Vasconcelos et al. 2006).

The stability of the species accumulation curve, as well as richness estimations, show that the sampling methods applied were appropriated to richness anuran species detection, and new records in this area are not expected. The “surveys at breeding sites” method was the most successful methodology concerning anuran species sampling in MDSP. This result reflects the anuran reproductive ecology, since all recorded species are dependent on lentic and/or lotic water bodies for reproduction (modes 1, 11, 13, 30 and modes 2 and 31 respectively, *sensu* Haddad and Prado 2005). The hydrological deficit recorded from March to October in inland São Paulo state reduce the water availability in the soil and for the vegetation (Juhász et al. 2007), and may affect the Atlantic anurans’ species which are dependent on humid microhabitats for reproduction, such as phytotelmatas and/or leaf litter (e.g. modes 5, 6, 8, 14, 21, 23, and 28 *sensu* Haddad and Prado 2005).

The MDSP anuran assemblage can be characterized as a mix of small groups of species with distribution associated with the Atlantic region (*Rhinella ornata*, *Hypsiboas faber*, and *Itapotihyla langsdorffii*), Chaco areas (*Leptodactylus chaquensis*), Cerrado (*Eupemphix nattereri*, *Hypsiboas albopunctatus*, and *H. lundii*) and of a greater group of species widely distributed in South America (e.g. *Dendropsophus minutus*, *D. nanus*, *Hypsiboas raniceps*, *H. punctatus*, *Leptodactylus*

*fuscus*, *L. cf. ocellatus*, *L. mystaceus*, *L. mystacinus*, *Physalaemus cuvieri*, *Pseudis platensis*, *Rhinella schneideri*, *Scinax fuscovarius*, and *Trachycephalus venulosus*) (Duellman 1999; Colli et al. 2002; Global Amphibian Assessment 2004; Frost 2008).

The majority of the MDSP anuran species is considered habitat generalist and tolerant to anthropic alterations (e.g. Brandão and Araújo 1998; Brandão 2002; Global Amphibian Assessment 2004; Brasileiro et al. 2005; Vasconcelos and Rossa-Feres 2005; Santos et al. 2007). From the 28 recorded species, only *Rhinella ornata*, *Hypsiboas faber*, *H. lundii*, and *Itapotihyla langsdorffii* can be considered as more associated to the forests and/or to their borders (Brandão and Araújo 1998; Izecksohn and Carvalho-e-Silva 2001a, Izecksohn and Carvalho-e-Silva 2001b; Baldissera et al. 2004; Eterovick and Sazima 2004, present study). Again, the seasonally dry climate probably limits the occurrence of anuran species typical of the wet Atlantic Forest in this region.

Specimens of *Hypsiboas faber* were recorded in forest border's water bodies, whereas *Rhinella ornata*, *Hypsiboas lundii*, and *Itapotihyla langsdorffii* were always recorded associated with very well preserved streams in MDSP. In addition, isolated information about the occurrence of these species in São Paulo state indicate that at least three of them (*Hypsiboas faber*, *H. lundii*, and *Itapotihyla langsdorffii*) do not occur in areas where the Mesophytic Semideciduous Forest was widely substituted with agricultural and pasture systems (Vasconcelos & Rossa-Feres 2005, Santos et al. 2007, Zina et al. 2007, authors' personal obs.). Therefore, the possibility that *Rhinella ornata*, *Hypsiboas lundii*, and *Itapotihyla langsdorffii* have been negatively affected by habitat degradation must be considered in future studies specifically delineated to access this question, since forest anurans with aquatic larvae are negatively affected by the disconnection of the forest remnants with their breeding sites, promoted by anthropic modifications (Becker et al. 2007).

### **Comparison with other locations**

The MDSP anuran composition, as well as those from other locations in the Mesophytic Semideciduous Forest, is more similar to those anuran assemblages recorded for Cerrado, Pantanal and even Pampa than those for the humid Atlantic Forest. The only exception to this pattern was Serra do Japi, which is grouped with typical Atlantic locations, since it represents a transition to humid forest (Leitão-Filho

1992), that reflects in anuran species composition (Bertoluci et al. 2007; Zina et al. 2007).

The similarity pattern of locations concerning anuran assemblage composition has been interpreted as a result of the geomorphologic formation of the studied areas (Dixo and Verdade 2006), of the climate or physiognomic similarity, and of geographical distance (Bastos et al. 2003; Brasileiro et al. 2005; Bertoluci et al. 2007). In fact, geographical distance influenced the similarity of faunistic composition among locations considered in this study. Groups with high similarity in anuran composition (e.g. CE2, FES4, FES10 and FES11; MDSP, CE7 and FES8) were geographically close, corroborating the reports of Bastos et al. (2003) and Bertoluci et al. (2007). However, this tendency was not statistically corroborated for a group of 11 remnants of Mesophytic Semideciduous Forest analyzed in São Paulo state (Zina et al. 2007), which may indicate that the sample universe considered in that study was insufficient to reach statistical significance, since the geographical distance effect seems to be present even in small spatial scales (see Dixo and Verdade 2006).

The pattern of faunistic similarity among locations was associated to the type of phytogeographic unit after taking out the geographical distance affect. The hypothesis matrix that most strongly correlated to the matrix of faunistic similarity was the one where the Mesophytic Semideciduous Forest areas hypothetically belonged to the Cerrado unit. This result indicates that the environmental conditions of a specific phytogeographic unit are very important to the anuran assemblage present in the area, such as the harsh climatic seasonality of the Cerrado, Pantanal and Mesophytic Semideciduous Forest.

It is possible that the Mesophytic Semideciduous Forests represent the relictual remnants of a formation that was widely spread in South America during the dry and cold Pleistocene period (Prado and Gibbs 1993; Pennington et al. 2000). Up to the present, in this type of forest, the ecological processes are highly seasonal (Pennington et al. 2000), which limits the activities of anuran species to the rainy season (e.g. Rossa-Feres and Jim 2001; Toledo et al. 2003; Zina et al. 2007; T.G. Santos unpublished data). In fact, Duellman (1999) considered this forest as part of the Cerrado-Caatinga-Chaco complex, instead of the Atlantic Forest Domain, due to the long dry season that is characteristic of these areas. In the Mesophytic

Semideciduous Forest, many anuran species show adaptations to xeric environments, such as deposition of eggs in foam nests and aestivation during the dry season. According to Duellman (1999), there are more anuran similarities between the Cerrado-Caatinga-Chaco complex and the Pampa than with the Atlantic Forest, which can also be influenced by seasonality. The Caatinga, Cerrado, Pantanal and Mesophytic Semideciduous Forest present rain with seasonal distribution (Arzabe 1999; Colli et al. 2002; Prado et al. 2005; present study), while areas in the Pampa are subject to marked temperature differentiation during the year (Santos et al. 2008).

Alternatively, a correlation of the faunistic similarity matrix with the hypothesis matrix considering the Mesophytic Semideciduous Forest as a unit that differs from other phytogeographic units, was also observed. This result is a consequence of the mixed nature of the anurans recorded in this phytogeographic unit (i.e. a mosaic of elements typically Amazonian, Atlantic, from the Cerrado and of wide distribution in South America) and not by the presence of endemic species, such as happens for lizards in central Brazil (Werneck and Colli 2006). On that account, the results for anurans do not corroborate Prado's hypothesis (2000) (i.e., that the Mesophytic Semideciduous Forest represents a new phytogeographic unit), due to the absence of exclusive species in this forest.

Our results indicate that the dry forest areas in São Paulo state do not have endemic species of anurans and have lower richness of species than the humid Atlantic Forest (see Heyer et al. 1990; Pombal and Haddad 2005). At least two non-exclusive hypothesis can be considered important to explain this pattern: i) the great distance in relation to the anuran diversification centers, such as the coastal mountains of the humid Atlantic Forest and, ii) the weather seasonality in inland São Paulo state (e.g. Barcha and Arid 1971; Rossa-Feres and Jim 2001; Santos et al. 2007), that limits the availability of humid microhabitats necessary for the reproduction of species with specialized reproductive modes (e.g. modes 5, 6, 8, 14, 18-23, 25, 27, 28, 32, 36, and 37, *sensu* Haddad and Prado 2005).

The first hypothesis is reinforced by the fact that areas of Mesophytic Semideciduous Forests close to coastal mountains have some typical species of humid Atlantic Forest (such as *Proceratophrys boiei* and *Hypsiboas prasinus* in the Mata São José locality; Zina et al. 2007), usually absent in those more distant (as in

this study). On the other hand, the second hypothesis is reinforced by the low diversity of reproductive modes recorded in the Mesophytic Semideciduous Forest (Zina et al. 2007; present study) and the prevalence of generalized modes (modes 1 and 2) and/or modes resistant to insulation/desiccation (modes 11, 13, 30, and 31), typically found in seasonal dry regions (e.g. Hödl 1990; Arzabe 1999; Prado et al. 2005; Vasconcelos and Rossa-Feres 2005; Santos et al. 2007). Climate rigor in the region can also be responsible for the prevalence of generalist species concerning habitat use in the studied area (see Santos et al. 2007).

The low species richness and the absence of endemism recorded in the Mesophytic Semideciduous Forest do not reduce their importance in anuran diversity maintenance. Forest remnants can be shelters and/or foraging sites for anurans (see example in Silva and Rossa-Feres 2007), as well as representing a stock of genetic variability. In this context, it is necessary to emphasize the necessity of phylogeographic studies, which are fundamental to the understanding of anuran dispersion and/or colonization history within the Mesophytic Semideciduous Forest. This type of study can be essential to determine specific conservation strategies for this forest, which is highly threatened by human activity.

## **Acknowledgments**

This study received financial support from the BIOTA/FAPESP program – The Biodiversity Virtual Institute ([www.biota.org.br](http://www.biota.org.br)). We thank Capes and CNPq for the doctorate fellowships given to T.G.S. and T.S.V., respectively; to IBAMA for the collection permit (02001.007052/2001); to the Morro do Diabo State Park (MDSP) direction and staff for the logistic support and stimulus and to everyone who participated in field work. We also thank Katia Kopp for suggestions on the manuscript. Special thanks are extended to Dr. Hussam Zaher (Fapesp: 02/13602-4) and Dr. Paula Eterovick for logistic support and improvements to the manuscript, respectively. CFBH thanks FAPESP and CNPq for financial support.

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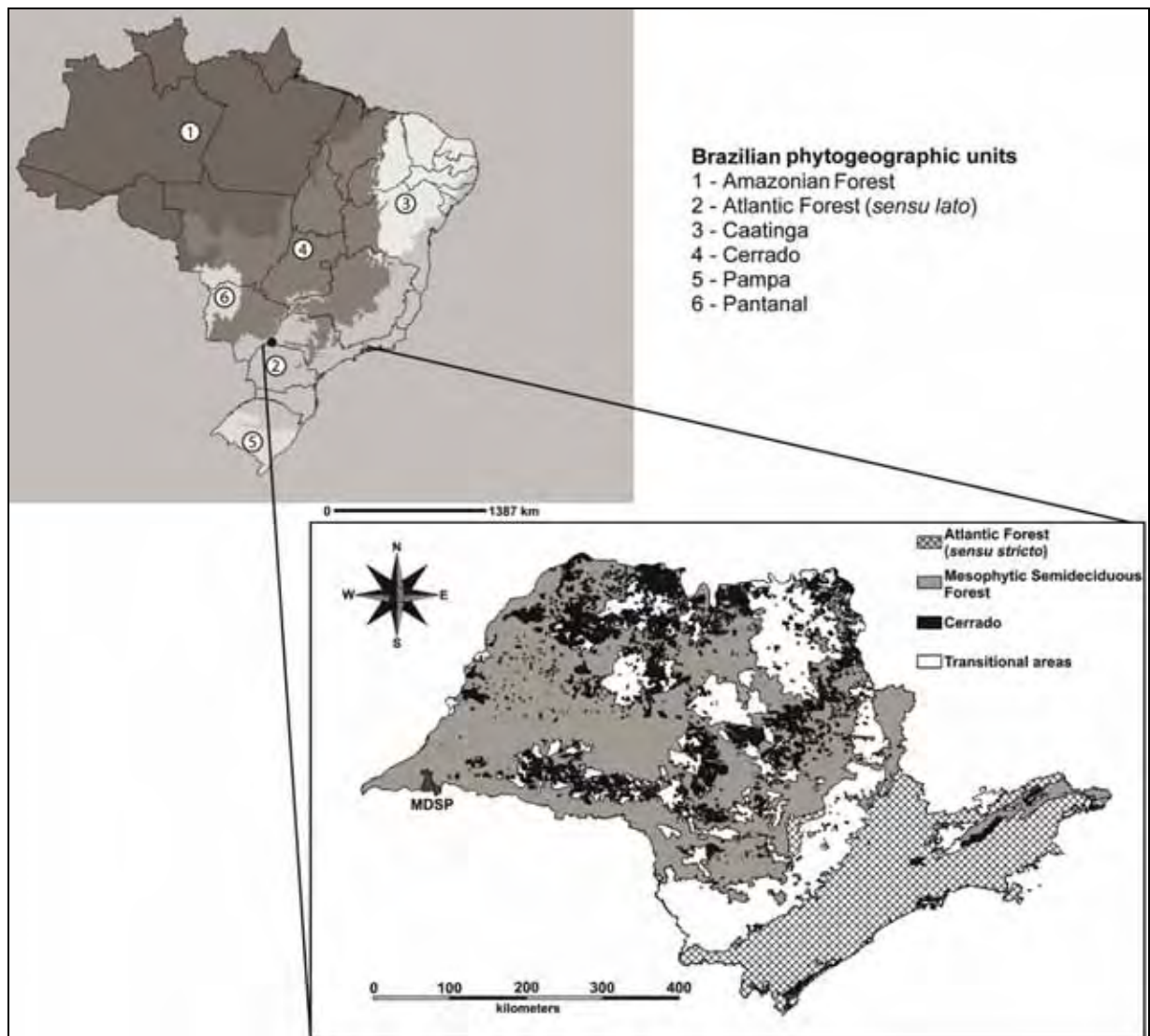
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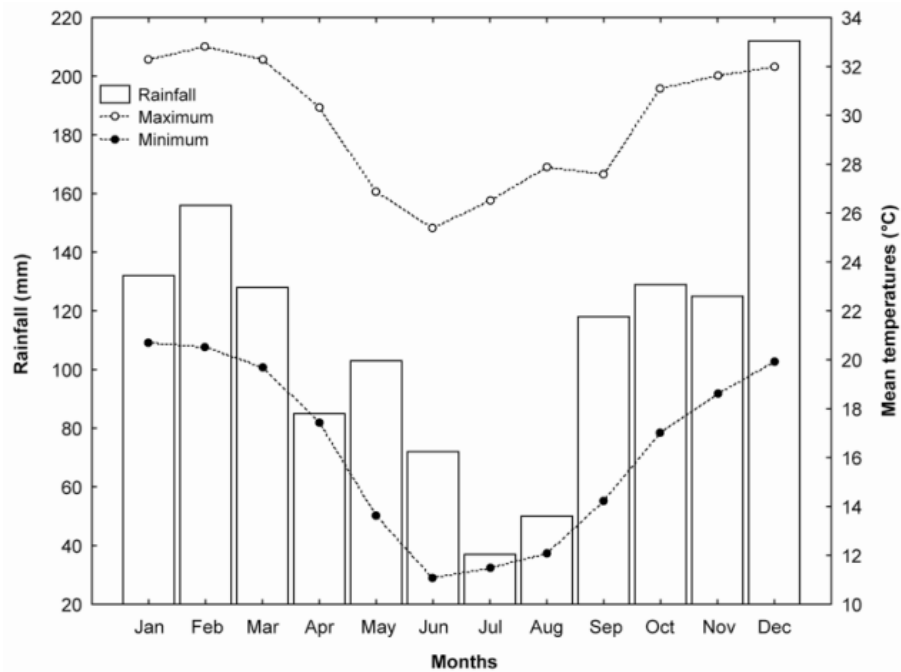
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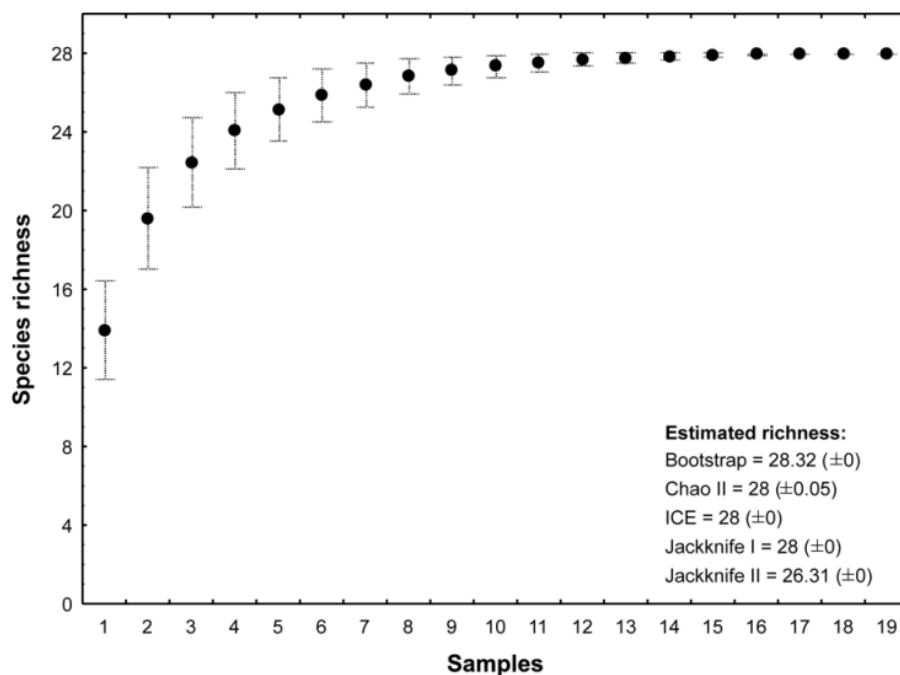
## FIGURES



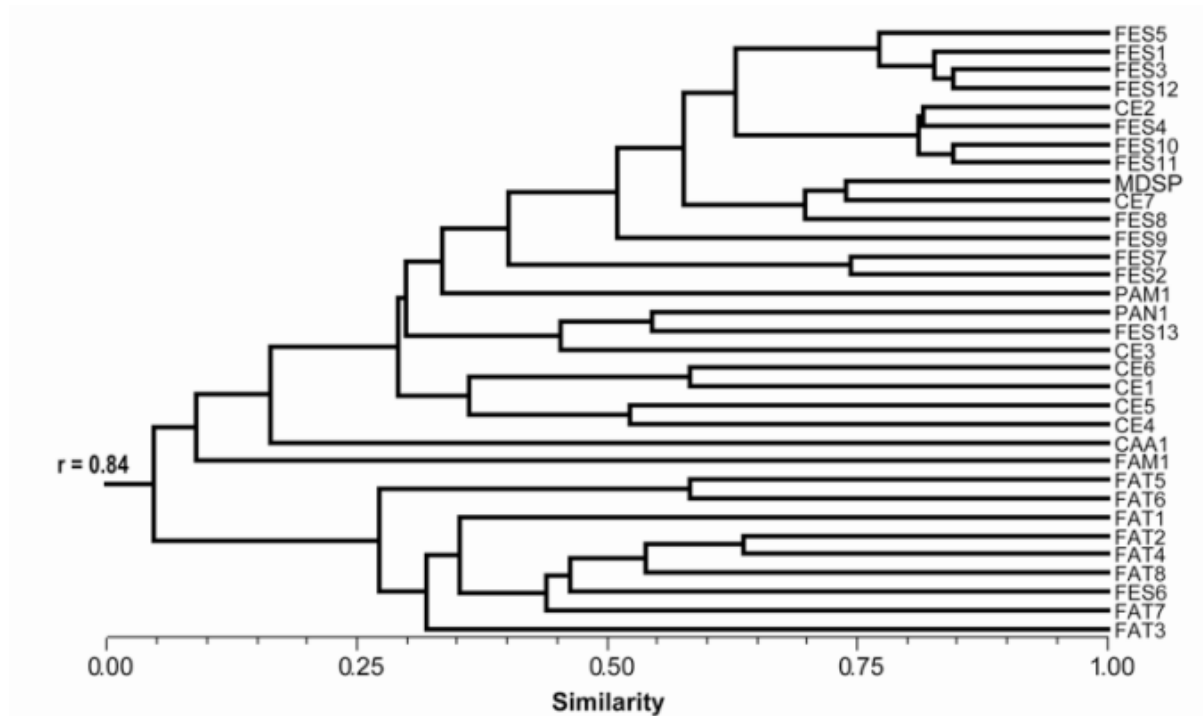
**Figure 1.** Phytogeographic units of Brazil, pointing out the state of São Paulo, and showing the location of Morro do Diabo State Park (MDSP).



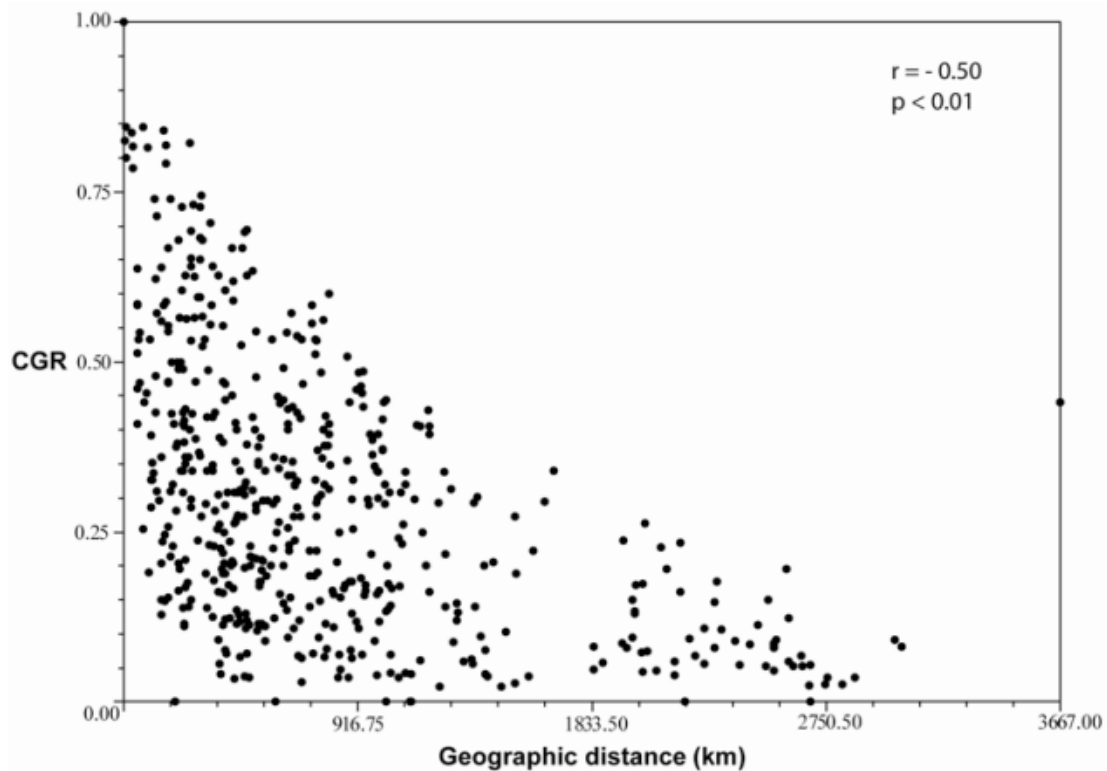
**Figure 2.** Historical rainfall distribution and minimum and maximum mean monthly temperatures recorded from 1977 to 2002 in MDSP, São Paulo state, Brazil. Source: Faria (2006).



**Figure 3.** Cumulative curve of species and richness estimators of anurans recorded in MDSP, São Paulo state, Brazil, from September 2005 to March 2007 based on sampling at breeding sites. The dots show the mean cumulative curve, generated by 500 randomized additions of samples, and the vertical bars indicate possible variation around the medium curve (confidence interval of 95%).



**Figure 4.** Similarity (CGR) in the taxonomic composition of the MDSP anuran assemblage with other areas of different phytogeographic units in the country.  $r$  = Cophenetic Correlation Coefficient. The abbreviations are in Table 1.



**Figure 5.** Dispersion diagram of the similarity matrix in the composition of the anuran assemblage (CGR) with the geographical distance matrix among the localities.  $p$  = significance level to Mantel's test ( $r$ ), using 5,000 Monte Carlo permutations.

## TABLES

**Table 1.** Phytogeographic units and respective localities of which anuran assemblages were compared to the one recorded in the MDSP, São Paulo state, Brazil. Sampling schedule (SC) = number of months (m) and days of field work (d); Record system (RS) = surveys at breeding sites (1), tadpole sampling (2), pitfall traps (3), visual survey (4), incidental encounters (5), and artificial shelters (6); Abbreviation (Ab). (?) = data not available and (\*) = localities intensively modified, with prevalence of pastures.

| Phytogeographic units                                                                                        | Localities                                                                     | SC       | RS         | Ab    |
|--------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|----------|------------|-------|
| Caatinga                                                                                                     | São José do Bonfim e Matureia, PB (Arzabe 1999)                                | 12m=72d  | 1,2        | CAA1  |
| Cerrado                                                                                                      | Estação Ecológica de Águas Emendadas, GO (Brandão and Araujo 1998)             | ?m=62d   | 1,4        | CE1   |
|                                                                                                              | Estação Ecológica de Itirapina, SP (Brasileiro et al. 2005)                    | 43m=302d | 1,3,5      | CE2   |
|                                                                                                              | Rio Manso, MT (Strüssmann 2000)                                                | 4m=40d   | 3,4,5      | CE3   |
|                                                                                                              | Parque Nacional da Serra da Canastra, MG (Haddad et al. 1988)                  | 4m=?d    | 1          | CE4   |
|                                                                                                              | Serra do Cipó, MG (Eterovick and Sazima 2004)                                  | ?m=205d  | 1,2        | CE5   |
|                                                                                                              | Floresta Nacional de Silvânia, GO (Bastos et al. 2003)                         | 60m=?d   | 1,3        | CE6   |
|                                                                                                              | Estação Ecológica de Assis, SP (Bertoluci et al. 2007)                         | 12m=24d  | 1,4        | CE7   |
| Mesophytic<br>Semideciduous<br>Forest or<br>Tropical Seasonal<br>Forests Region<br><i>sensu</i> Prado (2000) | Guararapes, SP (Bernarde and Kokubum 1999)                                     | 12m=?d   | 1,4        | FES1  |
|                                                                                                              | Londrina, PR (Machado et al. 1999)                                             | ?        | 1          | FES2  |
|                                                                                                              | Nova Itapirema, SP (Vasconcelos and Rossa-Feres 2005) *                        | 15m=25d  | 1,2        | FES3  |
|                                                                                                              | Floresta Estadual Edmundo Navarro de Andrade, SP (Toledo et al. 2003)          | 18m=52d  | 1, 4, 5    | FES4  |
|                                                                                                              | Santa Fé do Sul, SP (Santos et al. 2007) *                                     | 18m=18d  | 1,2        | FES5  |
|                                                                                                              | Serra do Japi, SP (Haddad and Sazima 1992)                                     | 12m=?d   | 1          | FES6  |
|                                                                                                              | Parque Estadual do Rio Guarani, PR (Bernarde and Machado 2001)                 | 12m=24d  | 1,4        | FES7  |
|                                                                                                              | Estação Ecológica de Caetetus, SP (Bertoluci et al. 2007)                      | 12m=24d  | 1,4        | FES8  |
|                                                                                                              | Mata de Santa Genebra, SP (Zina et al. 2007)                                   | ?        | 1,5        | FES9  |
|                                                                                                              | Mata São José, SP (Zina et al. 2007)                                           | 12m=?d   | 1          | FES10 |
|                                                                                                              | Distrito de Itapé (Zina et al. 2007)*                                          | ?        | 1          | FES11 |
|                                                                                                              | Icem, SP (Silva and Rossa-Feres 2007)*                                         | 12m=304d | 1,2,3,4, 6 | FES12 |
|                                                                                                              | Parque Nacional da Serra da Bodoquena, MS (Uetanabaro et al. 2007)             | 2m=24d   | 1,4        | FES13 |
| Amazonian Forest                                                                                             | Reserva Ducke, AM (Lima et al. 2006)                                           | ?        | ?          | FAM1  |
| Atlantic Forest<br><i>sensu stricto</i>                                                                      | Estação Ecológica da Boracéia, SP (Heyer et al. 1990)                          | ?        | ?          | FAT1  |
|                                                                                                              | Parque Estadual Intervales, SP (Bertoluci and Rodrigues 2002)                  | 12m=162d | 1          | FAT2  |
|                                                                                                              | Estação Ecológica Juréia-Itatins, SP (Pombal and Gordo 2004)                   | 10m=?d   | 1,4        | FAT3  |
|                                                                                                              | Ribeirão Branco, SP (Pombal and Haddad 2005)                                   | 24m=54d  | 1          | FAT4  |
|                                                                                                              | Município do Rio de Janeiro, RJ (Izecksohn and Carvalho-e-Silva 2001a)         | ?        | ?          | FAT5  |
|                                                                                                              | Floresta Nacional Mário Xavier, RJ (Izecksohn and Carvalho-e-Silva 2001b)      | ?        | ?          | FAT6  |
|                                                                                                              | Reserva Florestal de Morro Grande, SP (Dixo and Verdade 2006)                  | 4m=32d   | 3,4        | FAT7  |
|                                                                                                              | Parque Estadual Carlos Botelho, SP (Bertoluci et al. 2007, Moraes et al. 2007) | 24m=120d | 1,4,5      | FAT8  |
| Pampa                                                                                                        | Santa Maria, RS (Santos et al. 2008)                                           | 12m=48d  | 1,4        | PAM1  |
| Pantanal                                                                                                     | Corumbá, MS (Prado et al. 2005)                                                | 44m=176d | 1          | PAN1  |

**Table 2.** Anuran amphibians in the MDSP, São Paulo state, Brazil. Recording system (RS): pitfall traps with drift fences (PT), surveys at breeding sites (BS), sampling of tadpoles (T); reproductive modes (RM) *sensu* Haddad and Prado (2005).

| TAXA                                                      | RS        | RM    |
|-----------------------------------------------------------|-----------|-------|
| <b>Bufonidae</b> 02 species                               |           |       |
| <i>Rhinella ornata</i> (Spix 1824)                        | PT, T, BS | 2     |
| <i>Rhinella schneideri</i> (Werner 1894)                  | PT, T, BS | 1     |
| <b>Cycloramphidae</b> 01 species                          |           |       |
| <i>Odontophrynus americanus</i> (Duméril and Bibron 1841) | PT, BS    | 1     |
| <b>Hylidae</b> 14 species                                 |           |       |
| <i>Dendropsophus minutus</i> (Peters 1872)                | T, BS     | 1     |
| <i>Dendropsophus nanus</i> (Boulenger 1889)               | T, BS     | 1     |
| <i>Hypsiboas albopunctatus</i> (Spix 1824)                | T, BS     | 1/2   |
| <i>Hypsiboas faber</i> (Wied-Neuwiedi 1821)               | T, BS     | 4     |
| <i>Hypsiboas lundii</i> (Burmeister 1856)                 | BS        | 4     |
| <i>Hypsiboas punctatus</i> (Schneider 1799)               | T, BS     | 1     |
| <i>Hypsiboas raniceps</i> (Cope 1862)                     | T, BS     | 1     |
| <i>Itapotihyla langsdorffii</i> (Duméril and Bibron 1841) | BS        | 2?    |
| <i>Pseudis platensis</i> (Linnaeus 1758)                  | T, BS     | 1     |
| <i>Scinax berthae</i> (Barrio 1962)                       | T, BS     | 1     |
| <i>Scinax fuscomarginatus</i> (A. Lutz 1925)              | T, BS     | 1     |
| <i>Scinax fuscovarius</i> (A. Lutz 1925)                  | T, BS     | 1     |
| <i>Scinax cf. similis</i> (Cochran 1952)                  | T, BS     | 1     |
| <i>Trachycephalus venulosus</i> (Laurenti 1768)           | T, BS     | 1     |
| <b>Leiuperidae</b> 02 species                             |           |       |
| <i>Eupemphix nattereri</i> Steindachner 1863              | T, BS     | 11    |
| <i>Physalaemus cuvieri</i> Fitzinger 1826                 | PT, T, BS | 11    |
| <b>Leptodactylidae</b> 07 species                         |           |       |
| <i>Leptodactylus chaquensis</i> Cei 1950                  | T, BS     | 11    |
| <i>Leptodactylus fuscus</i> (Schneideri 1799)             | PT, T, BS | 30    |
| <i>Leptodactylus labyrinthicus</i> (Spix 1824)            | BS        | 13    |
| <i>Leptodactylus mystaceus</i> (Spix 1824)                | PT, BS    | 30/31 |
| <i>Leptodactylus mystacinus</i> (Burmeister 1861)         | T, BS     | 30    |
| <i>Leptodactylus cf. ocellatus</i> (Linnaeus 1758)        | BS        | 11    |
| <i>Leptodactylus podicipinus</i> (Cope 1862)              | PT, T, BS | 13    |
| <b>Microhylidae</b> 02 species                            |           |       |
| <i>Chiasmocleis albopunctata</i> (Boettger 1885)          | G, BS     | 1     |
| <i>Elaschistocleis bicolor</i> (Valenciennes 1838)        | G, BS     | 1     |

**Table 3.** Matrix correlations (r), using Mantel's test and Mantel Partial (5,000 Monte Carlo permutations). Matrices: Coefficient of Geographic Resemblance (CGR), of phytogeographic units, considering the Mesophytic Semideciduous Forest as part of the Atlantic unit (MS1), differing from the other phytogeographic units (MS2) and as part of the Cerrado unit (MS3). MGD represents the matrix of geographical distances.

| <b>Matrices</b>    | <b>r</b> | <b>p</b> |
|--------------------|----------|----------|
| CGR vs. MS1        | 0.26     | 0.01     |
| CGR vs. MS2        | 0.52     | <0.01    |
| CGR vs. MS3        | 0.68     | <0.01    |
| CGR vs. MDG        | - 0.50   | <0.01    |
| CGR vs. MS1 (-MGD) | 0.005    | 0.44     |
| CGR vs. MS2 (-MGD) | 0.43     | <0.01    |
| CGR vs. MS3 (-MGD) | 0.61     | <0.01    |



## CAPÍTULO 2

ANURAN TEMPORAL DISTRIBUTION IN SEASONALLY DRY FOREST OF THE SOUTHEASTERN  
BRAZIL: WHICH ENVIRONMENTAL VARIABLES BETTER EXPLAIN THE SPECIES BREEDING  
ACTIVITY?

# **ANURAN TEMPORAL DISTRIBUTION IN SEASONALLY DRY FOREST OF THE SOUTHEASTERN BRAZIL: WHICH ENVIRONMENTAL VARIABLES BETTER EXPLAIN THE SPECIES BREEDING ACTIVITY?**

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## **Abstract**

We analyzed the breeding activity of anurans in a subtropical remnant of seasonally dry forest in southeastern Brazil. We presented a new proposal of statistical approach for anuran calling activity in two scales (whole assemblage and breeding habitats), and determined the temporal overlap of species concerning the larval phase and the calling season. The higher species richness (calling males, tadpole occurrence, and froglet recruitments) occurs at the period of higher rainfall, and maximum temperature and photoperiods. Rainfall and photoperiod explained about 77% of calling activity of the whole assemblage, while rainfall alone in temporary habitats and photoperiod in permanent ones explained the calling season. We recorded high temporal overlap for calling males, but segregation for tadpoles, what probably occurred because of female temporal segregation. We also emphasized the necessity of a wide review of Neotropical patterns of anuran calling season using powerful statistical methods.

## **INTRODUCTION**

The breeding season of anurans is mainly related to local abiotic factors, such as seasonal distribution of rainfall, and availability and hydroperiod of breeding

habitats (Gottsberger & Gruber 2004, Wells 2007). Thus, breeding activity in wet temperate regions is concentrated in the warm period of the year, while in the tropical and subtropical regions it is related to the warm and rainy period (Duellman & Trueb 1994, Wells 2007). In addition, in aseasonal wet and warm tropical environments, it is expected to occur predominance of year-round breeding activity (Crump 1974), which enables opportunities for temporal segregation among species (Duellman 1978).

On the other hand, a concentrated breeding pattern is expected to be predominant in seasonally dry tropical environments, with high species overlapping at the rainy season (e.g., Rossa-Feres & Jim 1994, Prado *et al.* 2005, Vasconcelos & Rossa-Feres 2005, Santos *et al.* 2007).

Studies carried out at subtropical assemblages of South America do not indicate a single pattern of breeding activity, because the temperature alone (Bertoluci 1998, Bernarde & Machado 2001), the rainfall (Conte & Rossa-Feres 2007), the temperature and the rainfall (Conte & Machado 2005, Conte & Rossa-Feres 2006), or none of these variables (Pombal 1997, Bernarde & Anjos 1999) explain most of the anuran activity.

Currently, studies point out that temporal patterns of ectotherms for more austral assemblages of the subtropical region markedly differ from the previously reported on septentrional ones (see examples for anurans in Canavero *et al.* 2008, Both *et al.* 2008, and Santos *et al.* 2008, and for snakes in Di-Bernardo *et al.* 2007 and Winck *et al.* 2007, respectively). In fact, concerning the anuran assemblages, these studies reveal that neither rainfall and air humidity, nor temperature are good predictors of the calling activity (Both *et al.* 2008, Canavero *et al.* 2008, Santos *et al.* 2008), since the photoperiod and a sinusoidal model representing the latent circannual seasonality explain most of anuran calling activity (Both *et al.* 2008, Canavero *et al.* 2008, respectively).

In this context, the anuran breeding patterns in the Neotropical region require more studies. Here, we aimed: i) to describe the general breeding activity of anurans in a tropical remnant of seasonally dry forest through the calling season; ii) to determine the temporal occurrence of tadpoles and froglet recruitments; iii) to test which climatic variables best explain the calling pattern in two scales (whole assemblage and breeding habitats) using a new proposal of statistical approach; and

iv) to determine the temporal overlap of species regarding the larval phase and calling season.

## **MATERIAL AND METHODS**

### **Study area and data collection**

We carried out this study at Morro do Diabo State Park (MDSP), a remnant of tropical forest with approximately 33 845 ha located in the extreme western of the State of São Paulo (22°27'--22°40'S, 52°10'--52°22' W, and with altitudes ranging from 260 to 599.50 m a.s.l.), southeastern Brazil. The MDSP is covered by a mosaic of Mesophytic Semideciduous Forest (a type of seasonally dry tropical forest that belongs to the Atlantic Forest Domain) in different stages of regeneration, some small patches of Cerrado *sensu stricto*, and transitional forests (Durigan & Franco 2006). Ecological processes are considered strongly seasonal in this type of forest that usually receives less than 100 mm rainfall by at least 5--6 months throughout the year (Pennington *et al.* 2000). Regional climate is characterized as subtropical with dry winter and wet summer (Cwa type of Köppen's classification) (Leite 1998) and historical records of MDSP indicate mean temperature of 22 °C and annual rainfall ranging from 1 100 to 1 300 mm (Faria 2006). The length of the photoperiod in this area ranges from 10.47 h in winter (June) to 13.31 h in summer (December) (Observatório Nacional Brasileiro: <http://euler.on.br/ephemeris/index.php>).

We monitored six anuran breeding habitats in the MDSP: two streams, two temporary ponds, and two dams (Table 1). We sampled each selected breeding habitat from September 2005 to March 2007 and the sampling period was usually of six days per month. We based the records on acoustic survey of calling males and on tadpole collection. We registered males in calling activity by performing "survey at breeding sites" (*sensu* Scott & Woodward 1994), where we recorded the estimative of male abundance by walking slowly along the banks of the breeding habitats during the nocturnal period (usually from the sunset to midnight). Thus, the amount of time spent in each breeding habitat varied according to the size and the complexity of sites (Scott & Woodward 1994). We collected the tadpoles during diurnal period using a wire mesh dipnet in the banks of the breeding habitats (see Santos *et al.* 2007). We occasionally recorded data in other breeding habitats sampled inside and outside the MDSP (e.g., ephemeral ponds, dams, permanent swamps, floodplain

areas, and river creeks) and we also considered them in order to characterize the calling season and the tadpole temporal occurrence of anuran species.

We recorded temperature, rainfall, and photoperiod data monthly. We obtained the means of minimal temperature at the meteorological station of the Presidente Prudente municipality, located 100 km from the study site, and the monthly accumulated rainfall data from the MDSP climate station. We obtained the photoperiod data from the Observatório Nacional Brasileiro (<http://euler.on.br/ephemeris/index.php>), it was based on the mean of the monthly sampled days.

## Statistical analyses

### Analyses of the climatic variables as predictors of the anuran calling season

Most phenological studies of anuran breeding activity employ simple linear correlation methods (e.g., Pombal 1997, Bertoluci 1998, Bernarde & Anjos 1999, Bernarde & Kokubum 1999, Bertoluci & Rodrigues 2002, Gottsberger & Gruber 2004, Conte & Machado 2005, Conte & Rossa-Feres 2006, Santos *et al.* 2007) whereas a few use regression models (Toledo *et al.* 2003, Conte & Rossa-Feres 2007, Zina *et al.* 2007, Canavero *et al.* 2008). Here, we tested whether climatic variables can successfully predict the monthly richness of calling males in MDSP using Linear Multiple Regression analysis with aggregated data (Zar 1999) and with a panel data structure (Stock & Watson 2003). As far as we are concerned, this is the first time in the literature of phenological studies of anurans that this data structure is utilized. In addition, we attempted to minimize three major biases found usually unevaluated in previous studies: autocorrelation, heteroskedasticity, and multicollinearity.

In the first part of our statistical analysis, we performed a linear regression model with the aggregated data and computed the variation partitioning. In order to estimate adequately the coefficients and variances, we had to control the major forms of biases. The equation estimated was (Eq. 1):

$$Y_t = \alpha + \beta_1 P_t + \beta_2 R_t + v_t \quad (1)$$

Where  $Y_t$  is the richness of calling males,  $P_t$  is the photoperiod in period  $t$ ,  $R_t$  is the rain in period  $t$ ,  $v_t$  is the error term,  $\alpha$  is the intercept, and  $\beta_1$  and  $\beta_2$  are the regression coefficients.

The first problem addressed is the multicollinearity, which is the correlation among independent variables that may affect the correct estimate of regression model parameters and, consequently, produce spurious conclusions regarding the statistical model (Zar 1999). We computed a Pearson correlation matrix ( $r$ ) for pairs of climatic variables and then we excluded temperature from posterior analyses because it presented high correlation with rainfall ( $r = 0.60$ ) and photoperiod ( $r = 0.72$ ).

Other source of bias is the absence of homogeneity in the variance of original data, the heteroskedasticity. An assumption in regression analysis is that for all values of the independent variable, the variances of the corresponding values of the dependent variable are the same (i.e., there is homoskedasticity) (Legendre & Legendre 1998). We prevented possible heteroskedasticity data in the current study by computing the robust errors in the regression analyses (Wooldridge 2006).

Autocorrelation is a very general property of ecological variables, spatially and temporally observed, that may create problems in tests of statistical significance which require independence from the observations (including all the usual nonparametric and parametric tests) because it increases Type I errors (Legendre & Legendre 1998). Thus, in presence of autocorrelation, a significant correlation or regression coefficients can be, in fact, not significant (Legendre & Legendre 1998).

We employed the Durbin-Watson statistic ( $d$ ) to verify the presence of autocorrelation. It is a useful way to evaluate the presence of the first-order serial correlation (AR1) of residuals (i.e., whether or not residuals for adjacent cases are correlated, indicating that the observations or cases in the data file are not independent). Its value always lies between zero and four, and values near two usually indicate small residual autocorrelation. When the  $d$  statistic is substantially less than two, there is an evidence of positive autocorrelation and when it is substantially more than two it usually indicates negative correlation. However,  $d$  statistic also has zones of indecision where we can neither accept nor refute the presence of autocorrelation. We compared  $d$  values with both tabulated upper and lower critical values for  $d$  to determine how close to zero or four they are. Here,  $d$  statistic presented value close to two ( $d = 1.72$ ) and it lay in the zone that refutes first-order autocorrelation in the residuals concerning the  $d$  tabulated critical values.

We computed the variation partitioning in order to assess the following fractions of the whole data variation: i) variation explained by each climatic variable; ii) variation indifferently explained by all climatic variables joined; and iii) unexplained variation (see details in Legendre & Legendre 1998).

The second part of our statistical analysis was to perform a panel data model. Panel data analysis is a statistical method of studying a particular subject within multiple places, periodically observed over a defined time frame, sometimes defined as cross-sectional time series data (Stock & Watson 2003). Here, we considered six breeding habitats as subjects (Table 1) monitored over 18 periods, totaling 108 observations. The main characteristic of the panel data regression technique is the possibility to control omitted or uncontrolled/unknown variables (unobserved heterogeneity) which can affect the dependent variable (Baltagi 2005), a special problem in non-experimental research. In this context, the panel method represents adequate statistical approach to analyze aquatic environments, which are usually subjected to several uncontrolled variables in the field and constant over time (e.g., cover vegetation, shadow, banks structure, and bottom substrate).

Three basic panel analytic models exist concerning the assumed behavior of omitted variables over the cases (i.e., the monitored breeding habitats) and over the time: i) constant coefficient model (pooled), which do not consider omitted variables; ii) fixed effects model (FE), which consider that the omitted variables are correlated with explanatory variables, and iii) random effects model (RE), which consider that the omitted variables are not correlated with explanatory variables (see Greene 2003 for details). We expected that the best model had to be the random effect due to the presence of omitted variables, as mentioned above, and that we did not have to correlate those variables with climate variables (explanatory variables). In order to corroborate our hypothesis about which model is the most suitable to our dataset, we compared: 1) the pooled and random models by the Breusch-Pagan Lagrange multiplier test, and 2) the fixed and random models by the Hausman specification test (Wooldridge 2002). The tests confirmed that the most adequate model for our dataset is the random effects model ( $\chi^2 = 3.15$ ,  $p = 0.07$  to Breusch-Pagan Lagrange test, and  $\chi^2 = 3.07$ ,  $p = 0.99$  to Hausman test, respectively).

To understand the effect of each climate variables over the anuran calling activity in different types of breeding habitats we specified the random effect model as follow (Eq. 2):

$$Y_{it} = \alpha + \beta P_t W_{it} + \gamma R_t W_{it} + \mu_i + v_{it} \quad (2)$$

Where  $Y_{it}$  is the richness of calling males in the breeding habitat  $i$  in the period  $t$ ,  $P_t W_{it}$  is the interaction between photoperiod in  $t$  with the type of breeding habitat  $i$  in period  $t$ ,  $R_t W_{it}$  is the interaction between rain in  $t$  with the type of breeding habitat  $i$  in period  $t$ ,  $\alpha$  is the intercept, and  $\beta$  and  $\gamma$  are the regression coefficients. Using this construction the model has six variables for the first interaction and six variables for de second interaction. Note that in the random effect model the random heterogeneity specific to each cross-sectional unit  $\mu_i$  is uncorrelated with explanatory variables and constant through time. The intercepts vary across cross-sectional units; the slops are the same, and  $v_{it}$  is the error term (Greene 2003).

The presence of heteroskedasticity in panel data models can be detected by the likelihood ratio test. This test assumes homoskedasticity as the null hypothesis and the value of the LR statistic is 62.36 for the current data. Thus, we reject the null hypothesis and compute the panel model using robust standard errors (Wooldridge 2006).

Finally, the Wooldridge test evaluated the autocorrelation for panel models under the null hypothesis of no first-order serial correlation AR(1) in residuals from the regression (Wooldridge 2002). This statistical test evidenced AR(1) autocorrelation in the regression residuals ( $F = 12.27$  and  $p = 0.02$ ) and thus, we performed the feasible generalization least-squares (FGLS) regression model (Greene 2003). This method uses an estimated covariance matrix and consider individual characteristics (as traditional random effects model), in addition, it allows estimates in the presence of AR(1) autocorrelation and heteroskedasticity. Afterwards, we compared the results with those obtained with the traditional random effects generalized least-squares (RE GLS) regression model, without the control for AR(1) disturbance or heteroskedasticity.

We performed all analyses in Stata 9.2 software (StataCorp 2005). We carried out FGLS regression using the *xtgls* Stata's command. We controlled the autocorrelation in panel using the available command *corr(psar1)*, which assumes that serial correlation structure is unique for each panel. In the meantime, we treated

heteroskedasticity using the *panels(heteroskedastic)* command, which assumes different variances across panels (StataCorp 2005).

### Analyze of temporal guilds

We calculated the similarity in temporal distribution of calling males and tadpoles using the complement of Bray-Curtis index (Krebs 1999), and we represented the similarity matrix by the Non-Metric Multidimensional Scaling (NMDS) (Manly 1994). We considered guilds as species groups with similarity values > 60%. We performed the Stress statistic (S) to measure the goodness of fit between the similarities in the NMDS 2-d ordination space and the original similarities contained in the original matrices (Clark & Warwick 2001):  $S < 0.05$  gives an excellent representation of the original similarity matrix;  $S < 0.10$  corresponds to a good representation; and  $S < 0.20$  still gives a potentially useful 2-dimensional picture. We based the similarity matrices in log-transformed species abundance data ( $\log x+1$ ) and we performed the analyses using Primer 6.1 software (Clarke & Gorley 2006).

## **RESULTS**

### **Calling season and temporal distribution of tadpoles**

The highest numbers of species with calling males occur from September 2005 to March 2006 ( $14.29 \pm 4.15$  species) and from August 2006 to March 2007 ( $15.13 \pm 4.79$  species), which corresponded to the periods of the highest rainfall, maximum temperature, and maximum photoperiod (Fig. 1). We recorded the lowest calling activity from August to July 2006 ( $4.50 \pm 1.29$  species), a period with drier, colder, and shorter days (Fig. 1).

Multiple regression analysis corroborated the empirical observations, since rainfall and photoperiod explained about 77% of calling activity (23% to remain unexplained) (Table 2). Of the total variability in calling activity attributable to the climatic variables, rainfall alone contributed with 29%, photoperiod with 16%, and rainfall and photoperiod together indifferently explained 55% of it. However, the regression results in the panel analysis pointed out that climatic variables differently influenced the male calling activity through the groups of monitored breeding habitats: calling season was explained by rainfall in temporary ponds and by photoperiod in permanent dams and streams (Table 3). The regression models

showed congruent results, except for calling season activity in temporary ponds, which was predicted by rainfall and photoperiod in traditional regression model (without correction for autocorrelation and heteroskedasticity), while the corrected model evidenced significance only for rainfall (Table 3).

Calling season of the individual species corroborated the recorded pattern for the whole assemblage, because most species (approximately 79% of total) showed calling activity associated with the rainy seasons (Fig. 2). Males of some species in this group (e.g., *Eupemphix nattereri*, *Hypsiboas albopunctatus*, and *Leptodactylus fuscus*) also fortuitously called during one or two months in the dry season (onset and in the end of season) (Fig. 2).

Four species (approximately 14% of total) showed wide calling activity, including many months of the rainy and dry seasons: *Dendropsophus minutus*, *D. nanus*, *Scinax berthae*, and *Rhinella schneideri*. Calling activity of *Odontophrynus americanus* and *Rhinella ornata* was typically associated with the dry season (Fig. 2). Records of calling activity in whole MDSP region showed that most species (75% of total) have a higher calling season than those recorded only at the monitored breeding habitats (Fig. 2).

We sampled tadpoles of 21 species in MDSP region (Fig. 2). We failed to find tadpoles of six species (*Hypsiboas lundii*, *H. punctatus*, *Itapotihyla langsdorffii*, *Leptodactylus labyrinthicus*, *L. mystaceus*, and *L. ocellatus*). Most species (approximately 81% of the total) showed tadpole temporal occurrence associated to the rainy season (Fig. 2), but tadpoles of some species (*Leptodactylus podicipinus*, *Physalaemus cuvieri*, and *Scinax berthae*) also occurred fortuitously at the first month of the dry season (Fig. 2). Tadpoles of *Dendropsophus minutus*, *Hypsiboas albopunctatus*, *H. faber*, and *Rhinella ornata* presented similar temporal occurrence through the rainy and dry seasons (Fig. 2).

We recorded recruitment, characterized by the record of froglets, of ten species and it usually occurred from the middle to the end of the rainy season (Fig. 2). We recorded froglets of *Rhinella schneideri* at the onset of the rainy season and only three species presented recruitment in dry season (*Dendropsophus nanus*, *Hypsiboas raniceps*, and *Scinax fuscomarginatus*) (Fig. 2).

### Temporal guilds in the monitored breeding habitats

Similarity analysis yielded three species guilds concerning calling males with overlap above 60% (Fig.3A):

1) *Chiasmocleis albopunctata*, *Elachistocleis bicolor*, *Leptodactylus chaquensis*, *Physalaemus cuvieri*, *Scinax fuscovarius*, *S. similes*, and *Trachycephalus venulosus*, opportunistic species that always called after heavy rains. *Leptodactylus chaquensis* and *Trachycephalus venulosus* represented a subgroup into this species guild because the males called once in the 2005-2006 rainy season (the onset of season), while the other species called twice in this rainy season (in the onset and in the end of season).

2) *Dendropsophus nanus*, *Hypsiboas albopunctatus*, *H. raniceps*, *Leptodactylus podicipinus* and *Scinax fuscomarginatus*, species that called through the rainy seasons.

3) *Pseudis platensis* and *Rhinela schneideri*, species that called occasionally in the onset of the rainy season (November 2005).

Other nine anuran species (39% of total species) were not included in any guild (Fig.3A). Males of *Rhinella ornata* called only during the dry season (June to August 2006). Males of *Hypsiboas punctatus* called from the middle to the end of the rainy season (January to March/April 2006 and 2007). Males of *Scinax berthae* were opportunists and called after heavy rains in the rainy season and after occasional rains in the dry season. Males of *Dendropsophus minutus* called in the onset and in the end of the rainy season (October and November 2005 and February and March 2006). The only males of *Hypsiboas lundii* and *Leptodactylus labyrinthicus* recorded in the studied area called during some months of the rainy season (October and November 2005 and January 2006, for the former species, and October 2005 for the second species, respectively). Males of *Leptodactylus mystaceus* and *L. mystacinus* called in distinct months during the middle of the rainy season, while males of *Leptodactylus fuscus* called only in two months of the rainy season (October and December 2006).

For the larval phase, the similarity analysis revealed only one guild of species with overlap above 60% (Fig.3B): *Leptodactylus chaquensis* and *Scinax similis*, whose tadpoles occurred in the middle of the rainy seasons. Other seven anuran species (approximately 86% of total species) were not included in any guild (Fig.3B).

Tadpoles of *Leptodactylus mystacinus* and *L. podicipinus* presented temporal occurrence related to the rainy seasons, but with distinct peaks of abundance through the months (December 2006 for the former and November/December 2005, September 2006 and February 2007 for the second species). Occurrence of tadpoles of *Dendropsophus nanus* and *Scinax fuscomarginatus* was also related to the rainy season, but peaks of abundance occurred in December 2005 for the former species and in December 2005 and February 2007 for the second species. Tadpoles of *Elachistocleis bicolor* occurred throughout the rainy season, while those of *Chiasmocleis albopunctata* occurred at the end of the rainy season. Tadpoles of *Hypsiboas albopunctatus* occurred throughout the year, usually in higher abundance during the rainy seasons, while tadpoles of *H. raniceps* occurred along the rainy seasons. Tadpoles of *Scinax berthae* occurred in low abundance, punctually in the middle and in the end of the rainy season (November and December 2005 and April 2006). Tadpoles of *Rhinella schneideri* occurred at the onset of the rainy season (September 2006), while tadpoles of *R. ornata* occurred during the dry season and at the onset of the rainy season (July to October 2006).

## DISCUSSION

### Calling season and temporal distribution of tadpoles

In the last two decades, several studies addressed the primary importance of rainfall on the calling activity in assemblages of Neotropical anurans, usually in areas where this variable is seasonally distributed along the year (e.g., Rossa-Feres & Jim 1994, Arzabe 1999, Bernarde & Kokubum 1999, Gottsberger & Gruber 2004, Conte & Rossa-Feres 2007), but few of them tested the influence of photoperiod (see review in Both *et al.* 2008). We expected rainfall to be the major environmental variable explaining the calling activity at the whole studied anuran assemblage in the MDSP, because rainfall is seasonal in this area (Faria 2006). Thus, water availability constitutes a limiting factor for the breeding activity of most anuran species in the MDSP, since the assemblage is composed by species with reproductive modes dependent on water bodies (modes 1, 2, 4, 11, 13, 30, and 31 *sensu* Haddad & Prado 2005) (T G Santos unpubl. data). On the other hand, we did not expect a significant influence of the photoperiod in MDSP because, until now, this environmental variable has been considered important for Neotropical austral

assemblages where rainfall is not seasonal throughout the year (Both *et al.* 2008), and for several other animal groups in temperate regions in North hemisphere (see references in Bradshaw & Holzapfel 2007).

The photoperiod plays an important role in the synchronization of natural processes (including those linked to reproduction of ectotherms) because it gives a highly predictable cue of suitable environmental conditions (Gotthard 2001). In fact, length of favorable season declines with increasing latitudes, and the length of day in locations with latitude higher than 15° can provide a reliable seasonal cue enabling the start and end of physiological and developmental processes in seasonal life history of several groups (Bradshaw & Holzapfel 2007). Canavero *et al.* (2008) reported that a sinusoidal function representing seasonal predictable changes throughout the year explained most of the calling activity in austral assemblages, in Uruguay. In fact, the hypothetical latent variable represented by the sinusoidal function in Canavero's study is coincident with the circannual variation of the photoperiod in high latitudes, and therefore it seems to represent the same pattern that Both *et al.* (2008) recorded for Brazilian high latitude assemblages. Thus, the general pattern of anuran calling season recorded in the MDSP may be considered intermediary between austral assemblages, regulated primarily by photoperiod (Both *et al.* 2008), and the septentrional ones mainly regulated by rainfall (e.g., Donnelly & Guyer 1994, Rossa-Feres & Jim 1994, Bernarde & Kokubum 1999, Brasileiro *et al.* 2005, Conte & Rossa-Feres 2007). However, these studies in septentrional regions did not effectively test if photoperiod affects the reproductive activities of anuran assemblages in their respective areas. Hence, it is necessary to test this hypothesis based on a wide review of Neotropical patterns of anuran calling season including the photoperiod, as well as using powerful statistical methods.

The panel data analysis showed that the calling pattern is dynamic and related to specific characteristics of the monitored breeding habitats at MDSP. Therefore, rainfall in temporary breeding habitats and photoperiod in permanent ones explained the calling anuran season studied herein. Permanent ponds and streams are stable environments with water available throughout the year, while temporary ponds represent time-constrained resources for anuran breeding. For a Mediterranean area it was reported that permanent ponds have a breeding peak related to temperature, whereas reproductive success in temporary pond breeders is

determined by rainfall pulses (Richter-Boix *et al.* 2006). The pattern of anuran calling activity for temporary ponds in the present study agrees with the study of Richter-Boix *et al.* (2006), except for the contribution of the photoperiod that the RE traditional model indicated. However, we considered the results of RE model unrealistic due to the non-controlled autocorrelation and the heteroskedasticity in dataset. Besides, photoperiod could represent a less reliable cue in temporary ponds due to the inter-year variability in both start and longevity of the water phase (see example for insect diapauses in temporary pools in Williams 2006).

Mayhew's study (as cited in Williams 2006) summarized several adaptations of amphibians in seasonally dry habitats, such as lack of defined breeding season, breeding behavior initialized by rainfall, rapid congregation for reproduction, and fast development of eggs and larvae. In fact, these characteristics are congruent with our results because many species considered opportunistic concerning pond occupation pattern composed the anuran assemblages breeding at temporary ponds in MDSP (e.g., *Chiasmocleis albopunctata*, *Elachistocleis bicolor*, *Leptodactylus chaquensis*, *Physalaemus cuvieri*, *Scinax berthae*, *S. similis*, and *Trachycephalus venulosus*, T G Santos unpubl. data), whose males and females congregated only during few days at the breeding habitats, as soon as the ponds filled, at any time throughout the year. In addition, larval phase was short for anuran species breeding at temporary ponds (e.g., approximately two months for the microhylid *Elachistocleis bicolor*) (T S Vasconcelos unpubl. data), corroborating the statements of Mayhew's study.

On the other hand, in the present study, only the photoperiod predicted anuran calling activity in permanent breeding habitats. However, photoperiod was closely correlated to temperature, and both variables interact in a complex way in the control of anuran biological rhythms by triggering hormonal stimuli linked to reproduction (Saidapur & Hoque 1995), including calling activity (see Hatano *et al.* 2002, Both *et al.* 2008). In addition, variation in temperature turns it into a less predictable environmental cue than photoperiod itself (Gotthard 2001). Therefore, many anuran species that bred preponderantly at the permanent ponds and streams in MDSP (e.g., *Dendropsophus nanus*, *Hypsiboas albopunctatus*, *H. punctatus*, *H. raniceps*, and *Scinax fuscomarginatus*, T G Santos unpubl. data) are probably susceptible to changes in photoperiod because this variable is related to more suitable environmental conditions for breeding activities (e.g., higher temperatures

and/or air humidity). In this context, we suggest that future studies on anuran phenology must consider the breeding habitats individually due to their natural heterogeneities, and also test the influence of environmental variables measured for each breeding habitat on calling activity of distinct species guilds (e.g., the effect of air and soil humidity on species whose males vocalize perched or on the ground, respectively).

Rainfall pattern strongly affects the phenology of anuran reproduction (Gascon 1991) and juvenile recruitment is expected to occur at the end of rainy season (see Moreira & Lima 1991 and Donnelly & Guyer 1994). In the present study, the calling activity, with temporal distribution of tadpoles and events of froglet recruitments indicated that the dry period imposed restrictions to the breeding of most of the anuran species at MDSP, mainly for those dependent on the availability of temporary ponds.

### **Temporal guilds in the monitored breeding habitats**

The analysis of temporal guilds of adult males showed groups of species with high overlap in calling season, primarily related to the rainy periods. This result agrees with Basso's (1990) indication that anuran calling season is less important for partitioning of reproductive resources, and disagrees with what Eterovick and Sazima (2000) and Kopp and Eterovick (2006) reported, which found calling season segregation among species. The climatic equability (stable temperature, photoperiod, and rainfall through the year) and differences in activity cycles were considered as some of the factors promoting the low overlap among anuran species at Santa Cecília, Ecuador (Duellman 1978). Thus, the shorter period of suitable conditions for anuran activity (long and rainy days) throughout the year seems to result in high overlap among species during the calling season in seasonally dry areas (e.g., Vasconcelos & Rossa-Feres 2005, Santos *et al.* 2007), including the MDSP.

On the other hand, approximately 39% of the species presented temporal segregation (e.g., *Hypsiboas lundii*, *H. punctatus*, and *Rhinella ornata*) probably due to the climatic traits of the MDSP. The number of species overlapping in the time axis is usually higher for the assemblages monitored in localities with harsh dry season (i.e., localities without rainfall during some months) (Vasconcelos & Rossa-Feres 2005, Santos *et al.* 2007) than in always wet localities (Both *et al.* 2008, Santos *et al.*

2008). At the studied area, the dry season (from April to August) was less severe than what was reported by Vasconcelos and Rossa-Feres (2005) and Santos *et al.* (2007) in areas located in the northwestern region of São Paulo State, since rainfall occurred in all months during the dry period (although in low amount), enabling temporal segregation of anuran species in breeding activities at MDSP.

For the larval phase, the temporal analysis showed segregation among most of the species. The seasonality is considered the major axis in the resource partitioning among anuran larvae (Toft 1985) of periodically dry areas (Inger *et al.* 1986), and temporal partitioning is usually conspicuous within tadpole assemblages (see references in Duellman & Trueb 1994). However, studies conducted in the Neotropical region did not report a single pattern for temporal distribution of tadpoles. Some studies indicated the temporal segregation among species (e.g., Eterovick & Sazima 2000, Prado *et al.* 2005), while others emphasized that the segregation occurs only among guilds of species, evidencing high overlap at the intra-guild level (Vasconcelos & Rossa-Feres 2005, Santos *et al.* 2007), or further, they found high temporal overlap among species, but segregation within each guild because the species showed distinct peaks of abundance over the time (Rossa-Feres & Jim 1994).

A complex interaction of several factors such as environmental unpredictability, water chemistry, food availability, life history, predation risk, and competition (see references in Alford 1999 and Wells 2007) can influence the structure of tadpole assemblages. In addition, the temporal structure of tadpole assemblages can be a result of the temporal distribution of reproductive effort by adult anurans (Alford 1999). In the present study, the pattern of calling activity in the monitored breeding habitats was not a good predictor of tadpole occurrence because the male guilds did not correspond to the tadpole guilds. Therefore, other factors such as differential time that the females engaged in breeding activities can play a more important role preventing overlap in larval phase (and consequently explaining the temporal resource partitioning) than male calling activities, since the peaks of calling activity do not represent peaks of anuran reproduction.

## Acknowledgements

This study received financial support from the BIOTA/FAPESP program – The Biodiversity Virtual Institute ([www.biota.org.br](http://www.biota.org.br)). We thank Capes and CNPq for the PhD fellowships given to TGS and TSV, respectively. We say special thanks to Dr. Denise de C. Rossa-Feres and Dr. Hussam Zaher (Fapesp: 02/13602-4) for logistic support. We are grateful to the direction and staff of MDSP for logistic support and to everybody who participated in the field works. We also thank IBAMA for the collection permit (02001.007052/2001), and Marcia Spies for Primer use. CFBH thanks FAPESP and CNPq for financial support.

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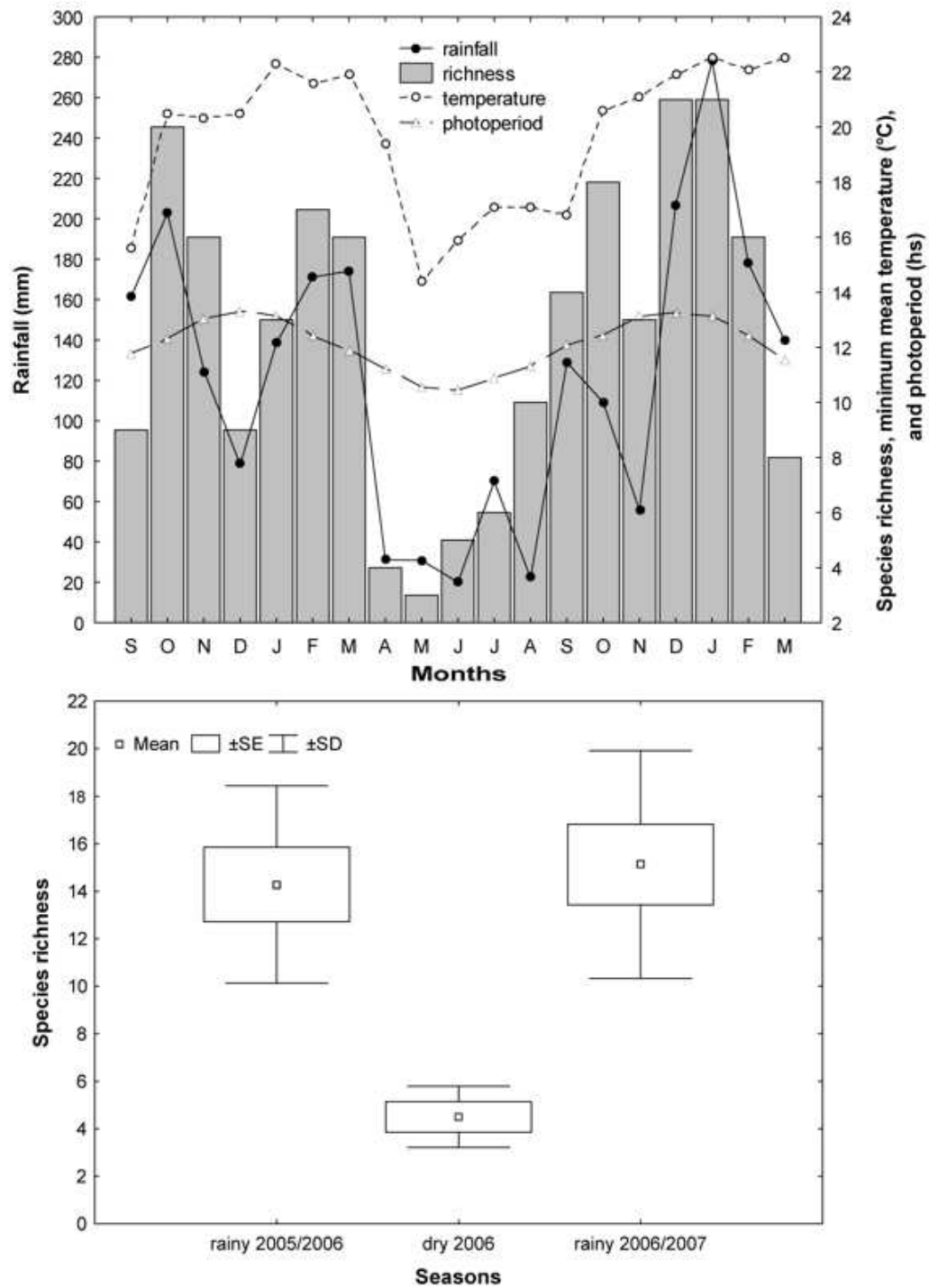
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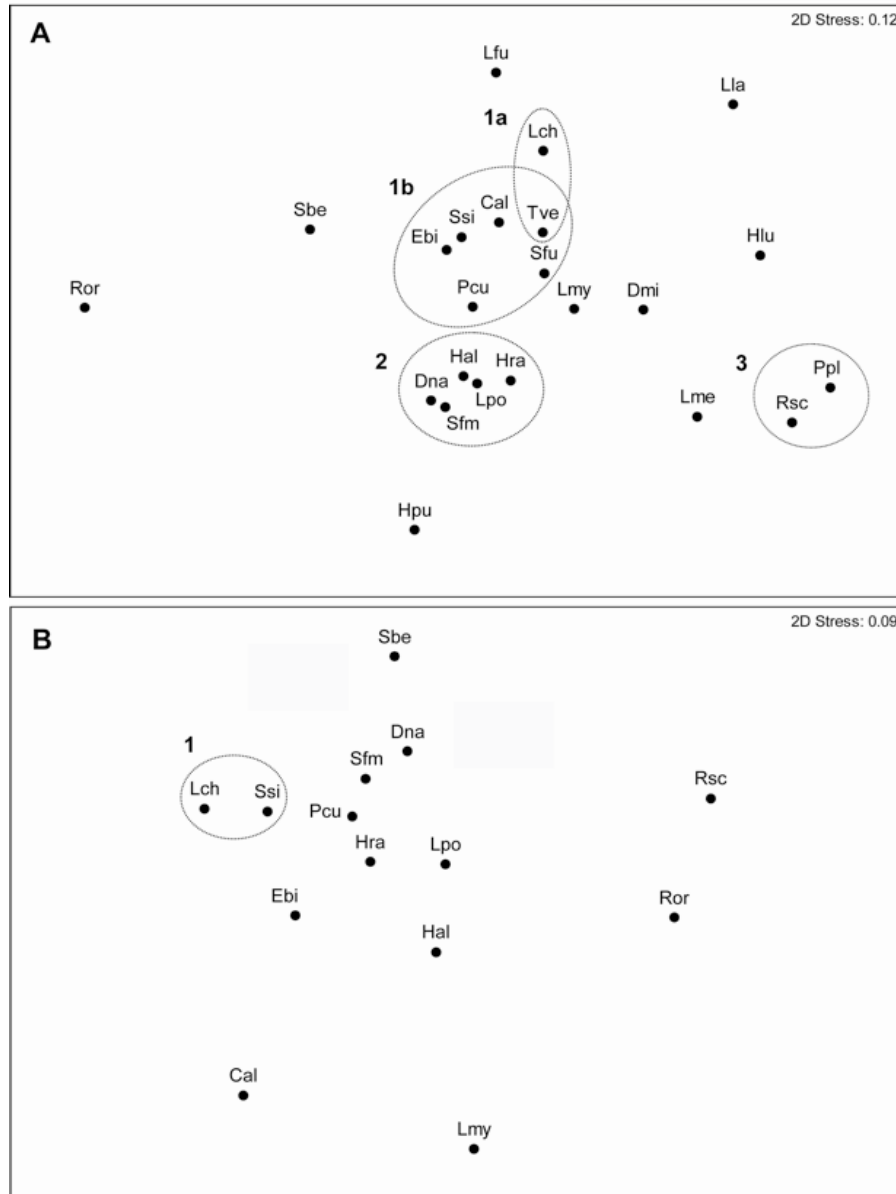
## FIGURES



**Figure 1.** Monthly distribution of climatic variables and richness of calling anurans (above) and richness box plots (below) of the anuran assemblage monitored at the MDSP, São Paulo State, Brazil.



**Figure 2.** Phenological distribution and richness of calling males (black bars), tadpoles (gray bars), and froglets (f) recorded at the MDSP, São Paulo State, Brazil. Asterisks represent records out of the monitored breeding habitats.



**Figure 3.** Temporal similarity of calling season (A) and larval phase (B) of anurans recorded at the MDSP, São Paulo State, Brazil. The ellipses and numbers represent the temporal guilds of species. Species: *Chiasmocleis albopunctata* (Cal), *Elachistocleis bicolor* (Ebi), *Dendropsophus minutus* (Dmi), *D. nanus* (Dna), *Hypsiboas albopunctatus* (Hal), *H. lundii* (Hlu), *H. punctatus* (Hpu), *H. raniceps* (Hra), *Leptodactylus chaquensis* (Lch), *L. fuscus* (Lfu), *L. labyrinthicus* (Lla), *L. mystaceus* (Lme), *L. mystacinus* (Lmy), *L. podicipinus* (Lpo), *Physalaemus cuvieri* (Pcu), *Pseudis platensis* (Ppl), *Rhinella ornata* (Ror), *R. schneideri* (Rsc), *Scinax berthae* (Sbe), *S. fuscomarginatus* (Sfm), *S. fuscovarius* (Sfu), *S. similis* (Ssi), and *Trachycephalus venulosus* (Tve).

## TABLES

**Table 1.** Characterization of six anuran breeding habitats monthly monitored (from October 2005 to March 2007) in the MDSP, São Paulo State, Brazil.

| Breeding habitats | Geographic coordinates       | Type   | Hydroperiod | Environment   |
|-------------------|------------------------------|--------|-------------|---------------|
| <b>PD1</b>        | 22°27'03.7"S<br>52°20'43.3"W | Dam    | Permanent   | Forest border |
| <b>PD2</b>        | 22°37'00.4"S<br>52°10'09.5"W | Dam    | Permanent   | Forest border |
| <b>PS1</b>        | 22°36'16.2"S<br>52°18'00.8"W | Stream | Permanent   | Inside Forest |
| <b>PS2</b>        | 22°28'30.8"S<br>52°20'30.9"W | Stream | Permanent   | Inside Forest |
| <b>TP1</b>        | 22°37'10.5"S<br>52°09'55.8"W | Pond   | Temporary   | Inside Forest |
| <b>TP2</b>        | 22°37'07.8"S<br>52°10'01.9"W | Pond   | Temporary   | Opened area   |

**Table 2.** Multiple regression between anuran calling activity and climatic variables recorded at the MDSP, São Paulo State, Brazil.

|                                         | Coef. (robust Std. Err.)             | <i>p</i>                     |
|-----------------------------------------|--------------------------------------|------------------------------|
| <b>Rainfall</b>                         | 0.045 (0.012)                        | 0.00                         |
| <b>Photoperiod</b>                      | 2.558 (0.965)                        | 0.02                         |
| <b>Constant</b>                         | -23.987 (10.486)                     | 0.04                         |
| <b>Total of observations</b>            | 18                                   |                              |
| <b>Adjusted <math>R^2 = 0.77</math></b> | <b><math>F_{2,16} = 48.58</math></b> | <b><math>p = 0.00</math></b> |

**Table 3.** Multiple regression analysis using random effects panel model without correction for autocorrelation and heteroskedasticity (RE) and random effects panel model with correction for autocorrelation and heteroskedasticity (RE+hetero+autocorr) to the anuran calling activity and climatic variables recorded at the MDSP, São Paulo State, Brazil. Monitored breeding habitats: permanent dams (P1 and P2), permanent streams (R1 and R2), and temporary ponds (T1 and T2). \*, significant at 1% and \*\*, at 5%. Value in parenthesis: z.

| Variables                    | Richness of calling males |         |                      |         |
|------------------------------|---------------------------|---------|----------------------|---------|
|                              | RE                        |         | RE hetero + autocorr |         |
| PD1 vs. rainfall             | 0.0032418                 | (0.78)  | 0.0053677            | (1.12)  |
| PD2 vs. rainfall             | 0.0048132                 | (1.40)  | 0.005753             | (1.71)  |
| PS1 vs. rainfall             | 0.0022191                 | (0.73)  | 0.0030742            | (1.16)  |
| PS2 vs. rainfall             | -0.005777                 | (-1.59) | -0.0043224           | (-1.37) |
| TP1 vs. rainfall             | 0.0328432*                | (2.78)  | 0.0339339*           | (3.00)  |
| TP2 vs. rainfall             | 0.0336221*                | (3.56)  | 0.0343311*           | (4.01)  |
| PD1 vs. photoperiod          | 0.7069396*                | (3.71)  | 0.5616284*           | (3.47)  |
| PD2 vs. photoperiod          | 0.66509*                  | (3.52)  | 0.53268*             | (3.42)  |
| PS1 vs. photoperiod          | 0.5760101*                | (3.06)  | 0.4468235*           | (2.94)  |
| PS2 vs. photoperiod          | 0.6407727*                | (3.42)  | 0.5040863*           | (3.26)  |
| TP1 vs. photoperiod          | 0.4487545**               | (2.04)  | 0.3144174            | (1.56)  |
| TP2 vs. photoperiod          | 0.3908593**               | (1.97)  | 0.2592415            | (1.43)  |
| <b>Constant</b>              | -6.321153*                | (-3.0)  | -4.80008*            | (-2.76) |
| <b>Total of observations</b> | 108                       |         | 108                  |         |
| <b>Wald Test</b>             | 289.06*                   |         | 96.44*               |         |



# CAPÍTULO 3

SPATIAL DISTRIBUTION OF ANURANS AMONG BREEDING SITES IN SOUTHEASTERN BRAZIL:

TESTING FOR INDICATOR SPECIES OF ENVIRONMENTAL HETEROGENEITY

# **SPATIAL DISTRIBUTION OF ANURANS AMONG BREEDING SITES IN SOUTHEASTERN BRAZIL: TESTING FOR INDICATOR SPECIES OF ENVIRONMENTAL HETEROGENEITY**

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## **Abstract**

We studied patterns of spatial distribution of anuran assemblages in Morro do Diabo State Park (MDSP), state of São Paulo, Brazil, using tests of null hypotheses. We recorded that spatial distribution of anuran species among breeding sites of MDSP differed from expected by chance and comprised three distinct anuran assemblages that were explained by the suit environmental variables of permanent streams, permanent dams, and temporary ponds. In addition, we recorded that 19 species (about 83% of total anuran species recorded in monitored sites) were indicators of environmental heterogeneity: three anuran species indicated permanent streams, four indicated permanent dams, and 12 anuran species indicated temporary ponds. We discussed spatial patterns of anuran species based in specific characteristics of breeding sites and in the ecology of the species, as well as pointed out implications of our study for conservation strategies of anurans at the Mesophytic Semideciduous Forest.

**Keywords:** anuran assemblages; Neotropics; similarity; null hypotheses; conservation.

## **INTRODUCTION**

Since 1950s, most ecologists have assumed that animal communities are not simply random assemblages of species (Wells 2007). Therefore, deterministic factors

were pointed out as responsible for variation in species diversity and composition along environmental and/or spatiotemporal gradients (Chase & Leibold 2003). However, according to recent Hubbell's Neutral Theory, the structure of assemblages (such as diversity and species composition) results from stochastic processes that are not influenced by species traits and/or environmental conditions (see details in Tilman 2004 & Chase 2007).

Some studies highlighted that Neotropical assemblages are weakly or not structured (see example in Eterovick & Barros 2003 and Afonso & Eterovick 2007 for anurans, and in França & Araújo 2007 for snakes), corroborating the Neutral Theory. On the other hand, partitioning of resources has been historically stressed in terms of both space and time for anuran assemblages (Crump 1971), where temporal axis has been considered the first dimension partitioned in the larval phase, and space in adult phase (Toft 1985). Several studies showed that similar habitats in relatively close distance often have a slightly different amphibian assemblages, since some species live in a variety of habitats while others can have more specialized habitat requirements (see references in Snodgrass et al. 2000 and Wells 2007). Therefore, most anuran assemblages appear to be structured, since differences in environmental conditions from site-to-site have explained differences in assemblage features, as richness and abundance of species (see references in Toft 1982). In addition, other factors such as biological interactions (e.g., predation and competition) and phylogenetic constraints are also considered important to explain structural patterns of assemblages (e.g., Zimmerman & Simberloff 1996, Eterovick & Sazima 2000, Eason Jr. & Fauth 2001, Werner et al. 2007).

According to Paton & Crouch (2002) and Bosch & Martínez-Solano (2003), local studies on breeding site preferences of amphibians can provide more accurate information for management purposes than studies in a wide scale, which can be affected by regional variations. In fact, spatial segregation in breeding sites occupancy has been recorded for adult and/or larval assemblages of anurans in local scale (e.g., Collins & Wilbur 1979, Basso 1990, Hero 1990, Gascon 1991, Eterovick & Sazima 2000, Bertoluci & Rodrigues 2002, Santos et al. 2007, Both et al. 2009), but unfortunately few studies have applied specific approaches to confirm assemblage structures. Thus, studies about spatial pattern of assemblages are urgent to delineate conservation strategies for anurans in ecosystems under strong

anthropogenic pressure, such as the Mesophytic Semideciduous Forests (MSF), the most fragmented and threatened ecosystem of the Brazilian Atlantic Domain (Viana & Tabanez 1996).

In the present study, we employed tests of null hypotheses to assess whether patterns of spatial distribution of anuran assemblages differ from a random distribution among aquatic breeding sites monitored at Morro do Diabo State Park (MDSP), one of the four largest remnants of MSF in Brazil (Durigan & Franco 2006). We also verified the existence of indicator anuran species of environmental heterogeneity in a local scale.

## **MATERIAL AND METHODS**

### **Study area and sampling procedures**

We carried out this study at Morro do Diabo State Park (MDSP), a remnant of seasonally dry tropical forest with approximately 33 845 ha located in southeastern Brazil (22°27'--22°40'S, 52°10'--52°22'W), where altitude ranges from 260 to 599.50 m a.s.l.) (a map of the studied area is given in Santos et al. 2009). MDSP is covered by a mosaic of Mesophytic Semideciduous Forest in different stages of regeneration, some small patches of Cerrado *sensu stricto* (savanna like vegetation), and transitional forests (Durigan & Franco 2006). Climate is characterized as subtropical with dry winter and wet summer (Cwa type of Köppen's classification) (Leite 1998), and historical records indicate mean temperature of 22 °C and annual rainfall ranging from 1,100 to 1,300 mm (Faria 2006).

We monthly monitored (from September 2005 to March 2007) six anuran breeding sites in the MDSP: two permanent streams (sections of 500 m in length), two permanent dams, and two temporary ponds (see a complete characterization in Table 1). Water physicochemical measurements were based in a mean of three samples, using Hach 2100P Turbidimeter (for turbidity) and YSI 556 Handheld Multiparameter (for other variables).

Permanent dams were located in borders of forests, presented great depth, low canopy cover, and bottom composed by clay and organic deposits. Dam waters showed low electric conductivity, intermediate oxygenation, and high richness of potential aquatic predators (insects, crustaceans, and fishes) (Table 1).

Permanent streams were located inside forests, presented bottom mainly composed by sand (PS2) and gravel (PS1), and backwaters with deposits of mud and organic matter. Stream waters were well oxygenated, with intermediate conductivity, and lower temperature than permanent dams and temporary ponds (Table 1).

Temporary ponds were shallow and presented bottom recovered by leaf litter and other detritus of terrestrial vegetation (as decaying grasses in TP2) that grows in dry pond basins. Waters of temporary ponds presented high conductivity, low dissolved oxygen, and low richness of potential aquatic predators (Table 1). In addition, temporary waters presented brownish coloration and high salinity, due to humic substances and evaporation, respectively (see references in Williams 2006).

We recorded monthly abundance of anuran species in each breeding site by performing “surveys at breeding sites” (*sensu* Scott & Woodward 1994), during the nocturnal period (usually from the sunset to midnight). The amount of time spent in each breeding site varied according to their size and complexity (Scott & Woodward 1994). Additional information regarding MDSP characterization and sampling schedule is available in Santos et al. (2009).

### **Statistical analyses**

We carried out an environmental representation of the monitored breeding sites at the MDSP by calculating Euclidean Distance index on abiotic and biotic quantitative measurements (i.e., water physicochemical features, size, canopy cover, and richness of potential aquatic predators). We based Euclidean Distances on transformed (root square) and normalized (by standard deviation) environmental variables, due to deviations of normality of original data and no comparable measurement scales (Clarke & Gorley 2006). In addition, we tested the existence of spatial patterns in the distribution of anuran assemblages among the six breeding sites monitored by computing similarity analysis (Bray-Curtis index) (see Krebs 1999). We based the similarity matrix in total abundance of anuran species in each breeding site. Abundance of each species in each breeding site was considered as the higher number of calling males recorded during the monitored months. We adopted this procedure to avoid overestimation of species due to recounting

individuals in a serial sampling schedule (see Gottsberger & Gruber 2004, Vasconcelos & Rossa-Feres 2005, Conte & Rossa-Feres 2006, Santos et al. 2007).

Then, we represented dissimilarity and similarity matrices by cluster analysis (UPGMA), and accessed statistical significances of genuine clusters performing the SIMPROF similarity profile test (Clarke & Gorley 2006). SIMPROF is a series of permutation tests of the null hypothesis that assumes that the samples are *a priori* unstructured (i.e., that the breeding sites are unstructured regarding environmental characteristics and/or anuran assemblages). This test is based on an expected profile shape of similarity/dissimilarity, obtained by permuting the entries for each variable 1000 times (i.e., species and/or environmental variables) across that subset of samples; this produces a null condition in which samples have no group structure. The 1000 permuted values are averaged to produce a mean profile, which is statically compared (999 times) with the real similarity profile by absolute distances (Phi) (Clarke & Gorley 2006). According to Clarke & Gorley (2006), whether environmental variables are responsible for structuring assemblages, it is expected that a plot based on environmental information groups the breeding sites in the same way as for species composition plot. Therefore, we looked for concordance among cluster plots of environmental characteristics and anuran assemblages, in order to explain spatial patterns of breeding sites use by anurans at the MDSP.

We also computed Principal Components Analysis (PCA) to represent together monthly samples of breeding sites and species. The purpose of this analysis is to capture as much as possible the variability of the original dataset in a low-dimensional solution, represented by orthogonal axes. Thus, most of the variance is accounted for the first two or three axes (see details in Clarke & Warwick 2001 and Manly 2008). We based PCA in a variance/covariance matrix, since we measured all variables in the same unit (i.e., abundance of anurans). Because our data set was composed by temporally serial samples (*sensu* Legendre & Legendre 1998) and because we were more interested in spatial than temporal structures, we used a covariable matrix in PCA representing time of sampling (i.e., sampling months as dummy variables) to minimize temporal effects in PCA solution.

Finally, we performed the Indicator Species Analysis (ISA) (Dufrêne & Legendre 1997) to test the existence of indicator species of the environmental heterogeneity. This method is based on a data matrix where there are data groups (a

*priori* established) that can be indicated by some species. According to McCune & Mefford (1999), each species get an indicator value (IV) of each group, which vary from 0 (no indication) to 100 (perfect indication). The null hypothesis in the ISA analysis considers that the maximum IV is not greater than the expected by chance. The indicator value is calculated using relative abundance and relative frequency of species across the sample units (considered herein as the monthly records of species abundance in each breeding site). Thus, a good indicator species of determined group must be frequent and abundant across samples in this group (Dufrêne & Legendre 1997). Established groups for ISA were considered based on the clusters indicated by SIMPROF and PCA analyses. Statistical significances of maximum value indicated for each group were performed using the Monte Carlo permutation test (5000 times). We based all analyses in log-transformed ( $\log x+1$ ) abundance of anuran species in order to down weight the contributions of quantitatively dominant species in the similarity analysis, and to linearize relationships in PCA analysis. SIMPROF and ISA analyses were performed using Primer-E 6.1 (Clarke & Gorley 2006) and PC-ORD 4.0 (McCune & Mefford 1999) software, respectively. PCA was performed using CANOCO 4.0 for Windows software (ter Braak & Smilauer 1998).

## RESULTS

We recorded a total of 23 anuran species in the six monitored breeding sites at the MDSP, and the number of anuran species by breeding site ranged from three (PS2) to 17 species (TP1) (Table 2). Anuran abundance ranged from 22 (PS2) to 316 individuals (TP1) (Table 2).

SIMPROF similarity profile test showed three consistent groups of breeding sites regarding environmental characteristics: permanent dams, permanent streams, and temporary ponds (Figure 1A). The first node separated temporary ponds from permanent dams and permanent streams (Figure 1A). The second node separated permanent streams from permanent dams (Figure 1A). In addition, the SIMPROF test also showed that anuran species differed from a spatial distribution expected by chance and clustered three consistent groups of breeding sites in the MDSP (Figure 1B). These groups correspond to the same groups evidenced in the analysis based on environmental features. The first node separated lotic (permanent streams) from

lentic environments (permanent dams and temporary ponds), whilst the second node separated lentic environments in permanent dams and temporary ponds (Figure 1B). The multivariate structure within the genuine clusters did not differ statistically (Figure 1AB).

PCA analysis showed congruent results with SIMPROF analysis, whereas the 2-dimensional solution of PCA accounted for 61.4% of total explained variance (Figure 2). Third and fourth axes account for few of the total explained variance (only 8.6% and 6%, respectively). PCA 1 (42.3%) showed tendency of segregation among samples of streams (mainly related to *Hypsiboas albopunctatus*), and samples of permanent dams and temporary ponds (mainly related to *Dendropsophus nanus*, *Hypsiboas raniceps*, *Leptodactylus podicipinus*, *Physalaemus cuvieri*, and *Scinax fuscomarginatus*). On the other hand, PCA 2 (19.1%) showed tendency of segregation among samples of permanent dams (mainly related to *Dendropsophus nanus* and *Scinax fuscomarginatus*) and samples of temporary ponds (mainly related to *Chiasmocleis albopunctata*, *Elachistocleis bicolor*, *Leptodactylus chaquensis*, *L. mystacinus*, *L. podicipinus*, *Physalaemus cuvieri*, *Scinax similis*, and *Trachycephalus venulosus*).

In fact, ISA results showed that the frequency of occurrence and abundance of 19 anuran species (about 83% of total species) is not at random when we adopted permanent dams, permanent streams, and temporary ponds as *a priori* groups in analysis (Table 3):

- *Hypsiboas albopunctatus*, *H. lundii*, and *Rhinella ornata* were indicator species of permanent streams.
- *Dendropsophus nanus*, *Hypsiboas punctatus*, *Scinax fuscomarginatus*, and *S. fuscovarius* indicated permanent dams.
- *Chiasmocleis albopunctata*, *Dendropsophus minutus*, *Elachistocleis bicolor*, *Hypsiboas raniceps*, *Leptodactylus chaquensis*, *L. fuscus*, *L. mystacinus*, *L. podicipinus*, *Physalaemus cuvieri*, *Scinax berthae*, *S. similis*, and *Trachycephalus venulosus* indicated temporary ponds.

Four anuran species were not species indicator of environmental heterogeneity: *Leptodactylus labyrinthicus*, *L. mystaceus*, *Pseudis platensis*, and *Rhinella schneideri* (Table 3).

## DISCUSSION

Our results indicated that anuran assemblages of MDSP were structured and related to the environmental heterogeneity of breeding sites, disagreeing with Hubbell's neutral model of communities (Hubbell 2001). Werner et al. (2007) also reported that amphibian assemblages of wetlands in Michigan, USA, deviated from the neutral model because spatial distribution of species across breeding sites differed from an expected random distribution, and that assemblage structure was also related to the environmental heterogeneity of ponds (i.e., mainly related to gradients of disturbance, productivity, and pond area). In fact, several studies around the world have shown that adults of anurans can actively choose breeding sites according to habitat characteristics (see references in Collins & Wilbur 1979, Eason & Fauth 2001, Bosch & Martínez-Solano 2003, Werner et al. 2007).

We expected a spatial structure of the anuran assemblages here studied, since the groups of monitored breeding sites at the MDSP (permanent dams, permanent streams, and temporary ponds) differed widely concerning environmental characteristics. For Neotropical anuran assemblages, studies also have pointed out differential utilization of breeding sites by adults and/or larvae of anurans (e.g., Basso 1990, Hero 1990, Gascon 1991, Eterovick & Sazima 2000, Bertoluci & Rodrigues 2002, Santos et al. 2007, Both et al. 2009), although few of them had effectively used statistical approaches for confirming spatial structure. In the present study, the remarkable degree of concordance between anuran assemblage and environmental plots (i.e., both group with very similar topology) indicated that the suit environmental variables have explained spatial patterns of anuran distribution among breeding sites (*sensu* Clarke & Gorley 2006).

We recorded by SIMPROF and PCA analyses a primary segregation of stream anuran assemblages at the MDSP. In fact, few anuran species ( $n = 7$ ) occurred in permanent streams (PS1 and PS2) and all of them occurred only in backwaters, except *Rhinella ornata* which also was recorded in riffles and running waters. The low anuran species richness recorded in streams was also reported in other localities of southern and southeastern Brazil (e.g., Bernarde & Anjos 1999, Bernarde & Machado 2001, Brasileiro et al. 2005, Vasconcelos & Rossa-Feres 2005), which may be resultant from three non exclusive hypotheses: i) phylogenetic constraints related to the historic process of colonization in South America (see

Zimmerman & Simberloff 1996); ii) pressure of aquatic predators (mainly fishes), considered strong in this type of environment (e.g., Gascon 1991, Magnusson & Hero 1991), and iii) morphological limitations in the larval phase (Gascon 1991).

We recorded through ISA analysis three anuran species as stream indicators: *Hypsiboas albopunctatus*, *H. lundii*, and *Rhinella ornata*. The former species is commonly associated to swamps (Brasileiro et al. 2005, Santos et al. 2007), have a generalized reproductive mode with deposition of eggs in ponds and exotrophic tadpoles developing in water (mode 1 *sensu* Haddad & Prado 2005), and it can be found in human settlements and in disturbed environments (e.g., Vasconcelos & Rossa-Feres 2005, Santos et al. 2007). In spite of the occurrence in streams of the MDSP, *H. albopunctatus* occurred only in backwaters of streams, very similar to swamps (i.e., in wide stream sections with mud bottom, under natural clearings).

*Hypsiboas lundii* is an anuran species typical from the Brazilian Cerrado (Frost 2008), totally dependent of gallery forests (see references in Brasileiro et al. 2005). Reproduction of this species is associated to permanent streams of primary and secondary forests (Global Amphibian Assessment 2004), and males usually construct basins in banks of streams, where eggs are deposited and tadpoles are carried out from basins after flooding caused by heavy rains (mode 4 *sensu* Haddad & Prado 2005) (Eterovick & Sazima 2004).

*Rhinella ornata* is distributed in the Atlantic forest (Frost 2008). It is a toad that deposits eggs in streams with exotrophic tadpoles developing in stream backwaters (mode 2 *sensu* Haddad & Prado 2005). Our observations indicate that males of this species called in standing backwaters and running waters of MDSP streams, highlighting that this toad species is dependent of forested habitats. In fact, recent study has demonstrated that populations of *R. ornata* undergoes genetic erosion, and consequently are under risk of local extinctions, due to habitat fragmentation of Atlantic Coastal Forest (Dixo et al. 2009). Here, we considered only *H. lundii* and *R. ornata* as truly associated to streams due to the restrict distribution of these species among breeding sites of MDSP (see detailed discussion in Santos et al. 2009).

In the current study, we recorded segregation between assemblages of permanent dams and temporary ponds at the MDSP. Anuran species richness in

permanent dams (PD1 and PD2) was intermediate among permanent streams and temporary ponds. In fact, dams have intermediate features between lotic and lentic environments, and it is harmful for the reproduction of many species, due to changes in physical and chemical features of water (Esteves 1998). These statements were corroborated by Both et al. (2008), since they recorded low diversity and high species dominance in dam environment monitored in southern Brazil. In the present study, dams were dominated by two anuran species (*Dendropsophus nanus* and *Scinax fuscomarginatus*), that have wide distribution in South America biomes and are typically well adapted to anthropogenic disturbances (Duellman 1999, Global Amphibian Assessment 2004, Frost 2008). These anuran species have the generalized reproductive mode (mode 1 *sensu* Haddad & Prado 2005).

In addition to *Dendropsophus nanus* and *Scinax fuscomarginatus*, other two anuran species were indicators of dams: *Hypsiboas punctatus* and *Scinax fuscovarius*. The former has wide distribution, comprising South and Central America (see Duellman 1999 and recent review in Vasconcelos et al. 2006), whilst *S. fuscovarius* present more southern distribution in South America (Frost 2008). Both species have the generalized reproductive mode (mode 1 *sensu* Haddad & Prado 2005) and can occur in distributed areas (Santos et al. 2007, 2008).

On the other hand, we recorded that temporary ponds (TP1 and TP2) supported high anuran species richness and were spatially structured, disagreeing with the stochastic pattern expected for habitats with low predictability (such as temporary water bodies) (Bonner et al. 1997). The pattern of high anuran species richness that we recorded in temporary ponds can be explained by the high productivity usually reported in temporary environments (see Williams & Feltmate 1992, Brönmark & Hansson 2005, and Williams 2006), as well as the absence or low abundance of aquatic predators (fishes and insects, respectively) in comparison with permanent ones (Heyer et al. 1975, Woodward 1983, Smith 1983, Skelly 1997), and the intermediate-disturbance hypothesis (see Both et al. 2009 for a full review).

In fact, comparisons of community composition between permanent and temporary waterbodies reveal relatively little overlap regarding to biota (see review in Williams 2006). High species richness has been reported in previous studies on Neotropical anuran assemblages of temporary ponds (e.g., Basso 1990, Zimmerman & Simberlof 1996, Toledo et al. 2003, Santos et al. 2007; Both et al. 2009) and

seems to be related to specific features of these environments, that make advantageous the colonization by several exclusive anuran species, mainly by opportunistic ones. In the present study, we reported many anuran species as indicators of temporary ponds, and our ecological data indicate that most of them (e.g., *Chiasmocleis albopunctata*, *Elachistocleis bicolor*, *Leptodactylus chaquensis*, *Physalaemus curvieri*, *Scinax similis*, and *Trachycephalus venulosus*) are opportunistic breeders with recruitment of calling males after heavy rains (Santos et al. unpubl. data).

Amphibians that use different types of ponds along the permanence gradient tend to have different life-history characteristics (Semlitsch et al. 1996), such as adaptations to deal with drying ponds. Therefore, anuran species that lay eggs in foam nests (e.g., leiuperids and leptodactylids) have an advantage to explore temporary environments due to protection of foam against desiccation of eggs and/or embryos (Heyer 1969, Downie 1988), and probably against wide fluctuations in the water levels, typical of temporary breeding sites (Vasconcelos & Rossa-Feres 2005, Santos et al. 2007). In addition, anuran species with generalized reproductive mode have high reproductive investments (large clutches), faster larval development, and can also show an evolutive response to explore unpredictable environments, as temporary ponds (Basso 1990). In our study, 42% of anuran species that were considered as indicators of temporary ponds deposited eggs in foam nests (mode 11: *Physalaemus curvieri* and *Leptodactylus chaquensis*, mode 13: *L. podicipinus*, and mode 30: *L. fuscus* and *L. mystacinus*), while the remaining 58% presented the generalized reproductive mode (mode 1: *Chiasmocleis albopunctata*, *Elachistocleis bicolor*, *Dendropsophus minutus*, *Hypsiboas raniceps*, *Scinax berthae*, *S. similis*, and *Trachycephalus venulosus*), highlighting that life-history characteristics of these species allow them to explore temporary sites.

The abundance and frequency of occurrence of four anuran species recorded at the MDSP did not differ from the expected by chance distribution (*Leptodactylus labyrinthicus*, *L. mystaceus*, *Pseudis platensis*, and *Rhinella schneideri*), and consequently did not indicate association with any breeding site. Three of them were rare at the monitored water bodies (*Leptodactylus labyrinthicus*, *Pseudis platensis*, and *Rhinella schneideri*), which becomes interpretations on spatial pattern distribution of these species unfeasible. On the other hand, *Leptodactylus*

*mystaceus* was a ubiquitous anuran species at the MDSP. We recorded males of this species calling within mud basins of stream banks and in temporary ponds, such as previously reported by Toledo et al. (2003). Reproduction of *L. mystaceus* takes place in sites seasonally flooded by heavy rains (Duellman 1978) and tadpoles are able to generate foam, probably as an adaptation to survive in places with unpredictable rainfall (Caldwell & Lopez 1989). Therefore, we believe that reproductive requirements of *L. mystaceus* seem to be more related to short hydroperiods of breeding sites (or parts of them, such as seasonally flooded banks of permanent streams at the MDSP) than to other environmental characteristics, such as lentic or lotic waters.

### **Concluding remarks**

We recorded that anuran assemblages at the MDSP were not a random set of species, since they were structured according to the three distinct groups of breeding sites (permanent dams, permanent streams, and temporary ponds). In addition, we also pointed out the existence of species indicators of the environmental heterogeneity, i.e., anuran species typical of each group of breeding sites. Regarding indicator species analysis (ISA), we observed lower values associated to anuran indicators of temporary ponds than for those of permanent dams and streams. This result seems to be related to reproductive pattern of anuran species, since ISA is based on relative abundance and relative frequency of species in the samples (Dufrêne & Legendre 1997). Therefore, explosive breeding species that occupied temporary ponds by short times contributed with few samples (i.e., low frequency of occurrence) in ISA analysis, decreasing the final indicator values. On the other hand, anuran species typical from permanent sites stayed in streams and dams for long times, increasing indicator values. Despite this possible bias in indicator values, we believed in the robustness of ISA results since we recorded higher number of anuran species as indicator of temporary ponds than permanent dams and streams. Ecological consistence of ISA can also be supported by other studies showing the same association of anuran species here indicated by ISA with temporary ponds or permanent breeding sites (see Brasileiro et al. 2005, Prado et al. 2005, Santos et al. 2007), although direct comparisons are limited due to the lack of similar statistical approaches.

Our results are relevant to conservation proposals, since Mesophytic Semideciduous Forests are the most fragmented and threatened ecosystem of the Atlantic Domain, and only 2% of this forest type remains in the state of São Paulo, as “islands of biodiversity” in an agricultural landscape (Viana & Tabanez 1996). Therefore, strategies of biodiversity conservation are urgently required for this forest type, and our results have significant implications in anuran conservation of remnants of Mesophytic Semideciduous Forest because highlighted the importance of heterogeneous breeding sites in the maintenance of high diversity in assemblages of anurans. In this way, the choice of areas for anuran conservation in this forest type must consider the presence of distinct breeding sites, such as lotic and lentic environments, as well as sites with different hydroperiods.

### **Acknowledgements**

This study received financial support from the BIOTA/FAPESP program – The Biodiversity Virtual Institute ([www.biota.org.br](http://www.biota.org.br)). We thank Capes and CNPq for the PhD fellowships given to TGS and TSV, respectively. We are grateful to IBAMA for the collection permit (02001.007052/2001), Fernando R. Carvalho and Luis G. Gorgatto for fish identification, Luciane Ayres Peres for crustacean identification and Marcia Spies for insect identification and Primer use. CFBH thanks FAPESP and CNPq for financial support.

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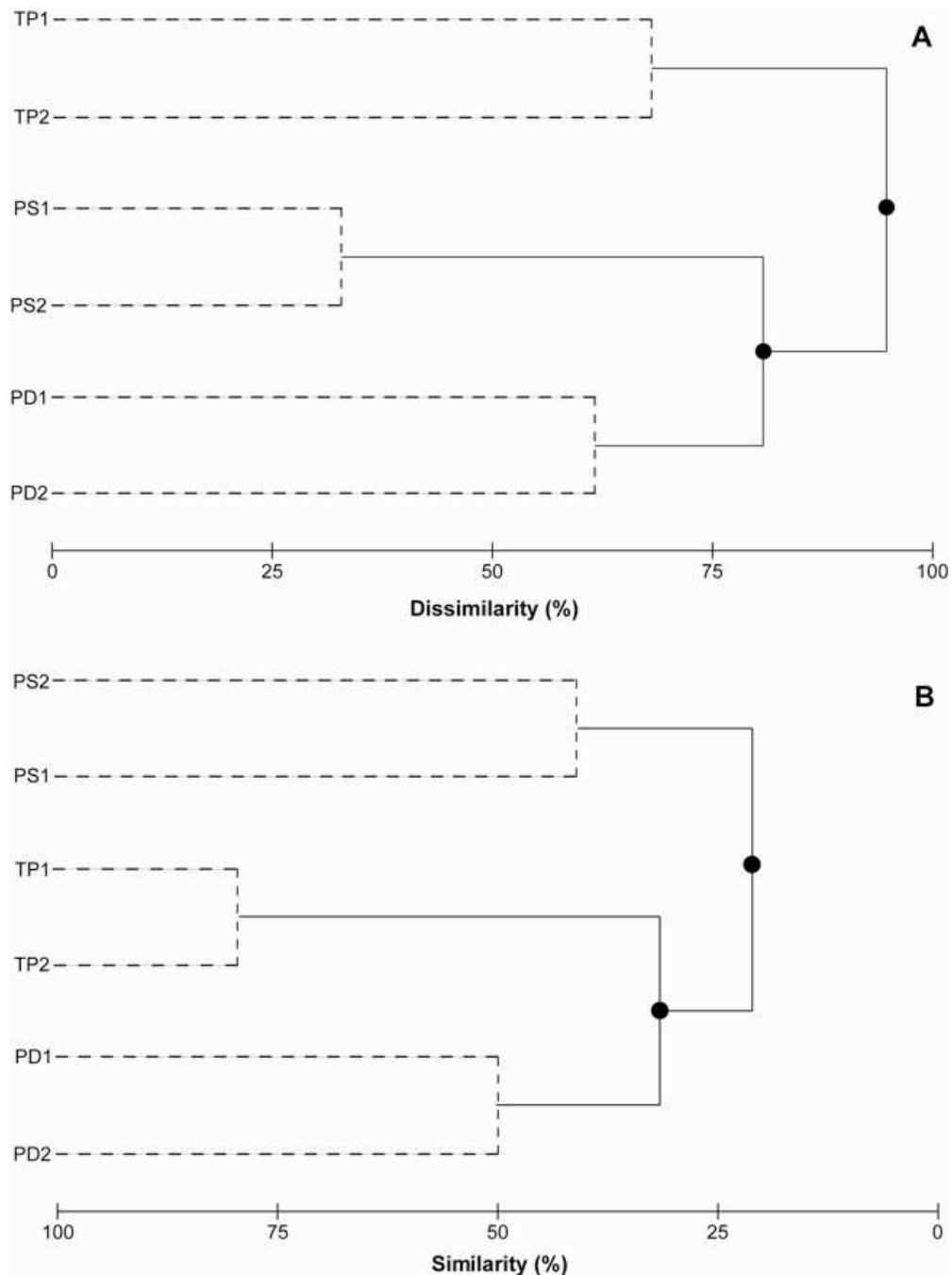
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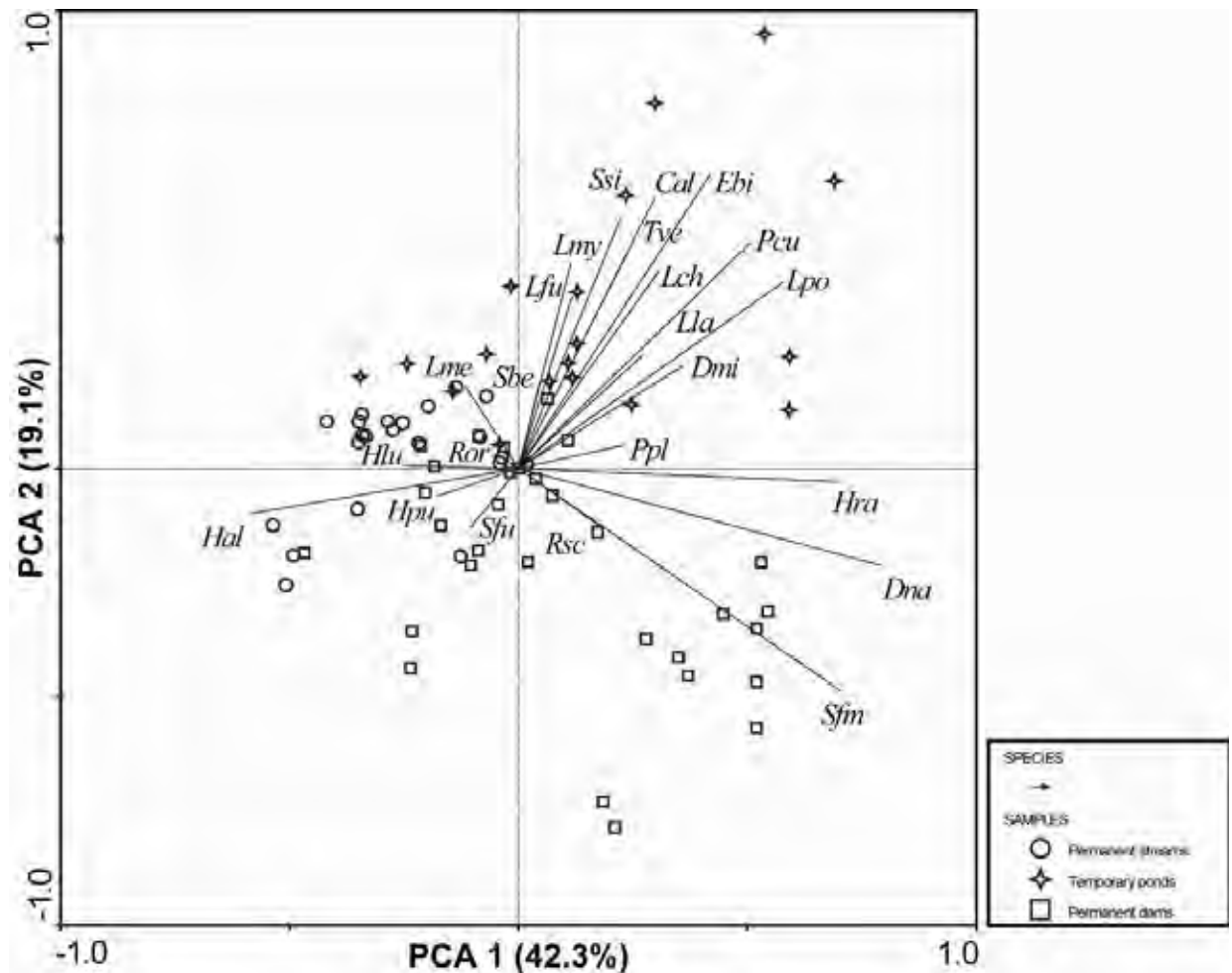
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## FIGURES



**Figure 1.** SIMPROF similarity profile test for environmental characteristics (Euclidian Distance index) (A) and anuran species composition (Bray-Curtis index) (B) recorded in the six monthly monitored breeding sites from October 2005 to March 2007 at the MDSP, state of São Paulo, southeastern Brazil: permanent dams (PD), permanent streams (PS), and temporary ponds (TP). Continuous lines indicate statistically consistent groups ( $p < 0.05$ ), whereas dotted lines indicates no statistical evidence for any structural pattern ( $p > 0.05$ ).



**Figure 2.** PCA ordination for 23 anuran species recorded in the six monthly monitored breeding sites (from October 2005 to March 2007) at the MDSP, state of São Paulo, southeastern Brazil. Species: *Chiasmocleis albopunctata* (Cal), *Dendropsophus minutus* (Dmi), *D. nanus* (Dna), *Elachistocleis bicolor* (Ebi), *Hypsiboas albopunctatus* (Hal), *H. lundii* (Hlu), *H. punctatus* (Hpu), *H. raniceps* (Hra), *Leptodactylus chaquensis* (Lch), *L. fuscus* (Lfu), *L. labyrinthicus* (Lla), *L. mystaceus* (Lme), *L. mystacinus* (Lmy), *L. podicipinus* (Lpo), *Physalaemus cuvieri* (Pcu), *Pseudis platensis* (Ppl), *Rhinella ornata* (Ror), *R. schneideri* (Rsc), *Scinax berthae* (Sbe), *S. fuscomarginatus* (Sfm), *S. fuscovarius* (Sfu), *S. similis* (Ssi), and *Trachycephalus venulosus* (Tve).

## TABLES

**Table 1.** Localization and environmental characterization of six breeding sites monitored at the MDSP, state of São Paulo, southeastern Brazil. Breeding sites: Permanent dams (PD), permanent streams (PS), and temporary ponds (TP); Water movement: running (r), inlet and outlet flow (io), and standing (s); Hydroperiod: number of months with water from September 2005 to March 2007; Origin: man-made (m), and natural (n); Edge type: flat (fl), and steep (st); Bottom substrate type: sand (sa), yellow clay (yc), gravel (gr), arenitic flagstone (af), accumulation of organic matter and mud (om), and cobble (co); Vegetation type: herbaceous (he), shrubby (sh), and arboreal (ar); Matrix vegetation type: disturbed open area (oa), Forest of Myrtaceae (fm), Forest of *Pinus* (fp), regeneration of Mesophytic Semideciduous Forest (rf), mature Mesophytic Semideciduous Forest (mf); Aquatic predator richness: numbers of families of insects and crustaceans, and number of species of fishes collected with dip nets through monthly sampling.

| Environmental<br>describers         | Breeding sites               |                              |                              |                              |                              |                              |
|-------------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
|                                     | PD1                          | PD2                          | PS1                          | PS2                          | TP1                          | TP2                          |
| <b>Geographic coordinates</b>       | 22°27'03.7"S<br>52°20'43.3"W | 22°37'00.4"S<br>52°10'09.5"W | 22°36'16.2"S<br>52°18'00.8"W | 22°28'30.8"S<br>52°20'30.9"W | 22°37'10.5"S<br>52°09'55.8"W | 22°37'07.8"S<br>52°10'01.9"W |
| <b>Altitude (m)</b>                 | 261                          | 264                          | 299                          | 299                          | 263                          | 259                          |
| <b>Water features</b>               |                              |                              |                              |                              |                              |                              |
| Electric conductivity (µS/cm)       | 18.33                        | 14.67                        | 24.33                        | 21                           | 49.33                        | 33.67                        |
| Dissolved O <sub>2</sub> [mg/L (%)] | 5.74 (72.3)                  | 3.39 (41.1)                  | 7.88 (90.4)                  | 8.04 (92.8)                  | 1.24 (15.43)                 | 1.25 (15.3)                  |
| pH                                  | 5.8                          | 4.67                         | 6.61                         | 5.68                         | 5.8                          | 5.52                         |
| Salinity (ppt)                      | 0.01                         | 0.01                         | 0.01                         | 0.01                         | 0.02                         | 0.01                         |
| Temperature (°C)                    | 27.08                        | 25.12                        | 22.1                         | 22.51                        | 26.52                        | 25.27                        |
| Turbidity (ntu)                     | 5.61                         | 26.57                        | 18.03                        | 5.12                         | 10.75                        | 46.74                        |
| Movement                            | io                           | s                            | r                            | r                            | s                            | s                            |
| <b>Size (m)</b>                     |                              |                              |                              |                              |                              |                              |
| Length                              | 200                          | 25                           | 500                          | 500                          | 70                           | 26                           |
| Width                               | 50                           | 5                            | 2.13                         | 2.7                          | 50                           | 27                           |
| Depth                               | >2                           | >2                           | 0.14                         | 0.26                         | 0.4                          | 0.48                         |
| <b>Hydroperiod (months)</b>         | 18                           | 18                           | 18                           | 18                           | 10                           | 8                            |
| <b>Origin</b>                       | m                            | m                            | n                            | n                            | n                            | n                            |

| Environmental<br>describers      | Breeding sites |            |                |            |            |            |
|----------------------------------|----------------|------------|----------------|------------|------------|------------|
|                                  | PD1            | PD2        | PS1            | PS2        | TP1        | TP2        |
| <b>Canopy cover (%)</b>          | <5             | <5         | 80             | 70         | 90         | <5         |
| <b>Edge type</b>                 | fl, st         | st         | fl, st         | fl, st     | fl         | fl, st     |
| <b>Bottom substrate type</b>     | yc, om         | yc, om     | sa, om, co, gr | sa, om, af | om, yc     | om, yc     |
| <b>Vegetation type</b>           |                |            |                |            |            |            |
| Aquatic                          | he             | he         | he             | he         | he, sh, ar | he, sh     |
| Edges                            | he, sh, ar     | he, sh, ar | he, sh, ar     | he, sh, ar | he, sh, ar | he, sh, ar |
| Matrix                           | fm, oa         | oa, rf, fp | mf             | fm         | oa, rf     | oa, rf     |
| <b>Aquatic predator richness</b> |                |            |                |            | 6          | 5          |
| Fishes                           | 4              | 3          | 4              | 3          | 1          | 1          |
| Insects and crustaceans          | 10             | 6          | 12             | 12         | 5          | 4          |

**Table 2.** Spatial distribution of anuran species among six monthly monitored breeding sites from October 2005 to March 2007 at the MDSP, state of São Paulo, southeastern Brazil: permanent dams (PD), permanent streams (PS), and temporary ponds (TP).

| <b>Species</b>                     | <b>PD1</b> | <b>PD2</b> | <b>PS1</b> | <b>PS2</b> | <b>TP1</b> | <b>TP2</b> |
|------------------------------------|------------|------------|------------|------------|------------|------------|
| <i>Chiasmocleis albopunctata</i>   | 0          | 0          | 0          | 0          | 7          | 15         |
| <i>Dendropsophus minutus</i>       | 0          | 0          | 0          | 0          | 5          | 1          |
| <i>Dendropsophus nanus</i>         | 50         | 20         | 0          | 0          | 45         | 25         |
| <i>Elaschistocleis bicolor</i>     | 0          | 0          | 0          | 0          | 30         | 25         |
| <i>Hypsiboas albopunctatus</i>     | 1          | 5          | 5          | 18         | 0          | 0          |
| <i>Hypsiboas lundii</i>            | 0          | 0          | 0          | 1          | 0          | 0          |
| <i>Hypsiboas punctatus</i>         | 0          | 12         | 0          | 0          | 0          | 0          |
| <i>Hypsiboas raniceps</i>          | 6          | 0          | 0          | 0          | 20         | 8          |
| <i>Leptodactylus chaquensis</i>    | 0          | 0          | 0          | 0          | 7          | 1          |
| <i>Leptodactylus fuscus</i>        | 0          | 0          | 0          | 0          | 2          | 4          |
| <i>Leptodactylus labyrinthicus</i> | 0          | 0          | 0          | 0          | 1          | 0          |
| <i>Leptodactylus mystaceus</i>     | 0          | 0          | 3          | 0          | 1          | 0          |
| <i>Leptodactylus mystacinus</i>    | 0          | 0          | 0          | 0          | 20         | 8          |
| <i>Leptodactylus podicipinus</i>   | 10         | 2          | 6          | 3          | 100        | 30         |
| <i>Physalaemus cuvieri</i>         | 8          | 0          | 7          | 0          | 32         | 15         |
| <i>Pseudis platensis</i>           | 0          | 0          | 0          | 0          | 1          | 0          |
| <i>Rhinella ornata</i>             | 0          | 0          | 13         | 0          | 0          | 0          |
| <i>Rhinella schneideri</i>         | 3          | 0          | 0          | 0          | 0          | 0          |
| <i>Scinax berthae</i>              | 0          | 5          | 0          | 0          | 17         | 11         |
| <i>Scinax similis</i>              | 0          | 0          | 0          | 0          | 2          | 30         |
| <i>Scinax fuscomarginatus</i>      | 60         | 12         | 0          | 0          | 0          | 0          |
| <i>Scinax fuscovarius</i>          | 0          | 0          | 0          | 0          | 8          | 3          |
| <i>Trachycephalus venulosus</i>    | 0          | 0          | 1          | 0          | 18         | 5          |
| <b>Species abundance</b>           | <b>138</b> | <b>56</b>  | <b>35</b>  | <b>22</b>  | <b>316</b> | <b>181</b> |
| <b>Species richness</b>            | <b>7</b>   | <b>6</b>   | <b>6</b>   | <b>3</b>   | <b>17</b>  | <b>14</b>  |

**Table 3.** Species Indicator Analysis (ISA) for spatial distribution of anuran species in the six monthly monitored breeding sites from October 2005 to March 2007 at the MDSP, state of São Paulo, southeastern Brazil. Groups: 1) permanent streams; 2) permanent dams; and 3) temporary ponds. Indicator values (IV) and statistical significance (p). ns =  $p > 0.05$ .

| Species                            | Group | IV   | p    |
|------------------------------------|-------|------|------|
| <i>Chiasmocleis albopunctata</i>   | 3     | 27.8 | 0.00 |
| <i>Dendropsophus minutus</i>       | 3     | 27.8 | 0.00 |
| <i>Dendropsophus nanus</i>         | 2     | 36.2 | 0.03 |
| <i>Elaschistocleis bicolor</i>     | 3     | 44.4 | 0.00 |
| <i>Hypsiboas albopunctatus</i>     | 1     | 52.9 | 0.00 |
| <i>Hypsiboas lundii</i>            | 1     | 13.6 | 0.04 |
| <i>Hypsiboas punctatus</i>         | 2     | 21.9 | 0.01 |
| <i>Hypsiboas raniceps</i>          | 3     | 33.9 | 0.01 |
| <i>Leptodactylus chaquensis</i>    | 3     | 16.7 | 0.02 |
| <i>Leptodactylus fuscus</i>        | 3     | 16.7 | 0.02 |
| <i>Leptodactylus labyrinthicus</i> | 3     | 5.6  | ns   |
| <i>Leptodactylus mystaceus</i>     | 1     | 11.3 | ns   |
| <i>Leptodactylus mystacinus</i>    | 3     | 38.9 | 0.00 |
| <i>Leptodactylus podicipinus</i>   | 3     | 65.1 | 0.00 |
| <i>Physalaemus cuvieri</i>         | 3     | 53.2 | 0.00 |
| <i>Pseudis platensis</i>           | 3     | 5.6  | ns   |
| <i>Rhinella ornata</i>             | 1     | 13.6 | 0.04 |
| <i>Rhinella schneideri</i>         | 2     | 3.1  | ns   |
| <i>Scinax berthae</i>              | 3     | 22   | 0.02 |
| <i>Scinax fuscomarginatus</i>      | 2     | 38.7 | 0.00 |
| <i>Scinax fuscovarius</i>          | 2     | 18   | 0.02 |
| <i>Scinax similis</i>              | 3     | 27.8 | 0.00 |
| <i>Trachycephalus venulosus</i>    | 3     | 21.2 | 0.01 |



## CAPÍTULO 4

CALLING SITE USE AMONG ANÜRANS IN TEMPORARY PONDS IN SOUTHEASTERN BRAZIL:

PARTITIONING OR OVERLAP?

## **CALLING SITE USE AMONG ANURANS IN TEMPORARY PONDS IN SOUTHEASTERN BRAZIL: PARTITIONING OR OVERLAP?**

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### **Abstract**

We studied calling site usage of anuran species in two temporary ponds (TP1 and TP2) at Morro do Diabo State Park (MDSP), Southeastern Brazil. We aimed i) to test if anuran species use different calling sites during peaks of anuran breeding activity in temporary ponds; ii) to test if there is relationship between calling site usage and phylogenetic distance of anuran species; iii) to test if anuran species use different calling sites in the ponds; and iv) to found the subset of calling sites variables that better discriminate anuran species. Overall the tests rejected the null hypothesis of no differences among vocalization sites of anuran species and pair-wise comparisons showed that males of most pairs of species (96%) used distinct sites for vocalization activities. Comparisons also showed that most species tested (67%) differed regarding calling sites between the studied ponds (i.e., presented high intra-specific average dissimilarity). The best combination of variables discriminating anuran species regarding male calling sites was type of substratum, pond micro-relief, and pond type. In addition, the type of substratum, pond micro-relief, water depth, and perching height alone also presented discriminatory power. The similarity patterns in calling site use have no relation with phylogenetic distance among anurans species. Calling site partitioning can occur even in highly opportunistic aggregations of anurans, such as in temporary ponds. The patterns of calling site use here reported can be explained mainly by environmental heterogeneity of ponds, as well as by the ecological plasticity of anuran species.

**Keywords:** anuran assemblages, similarity, null hypothesis, General Discriminant analysis, best predictors.

## INTRODUCTION

Microhabitat preferences are considered adaptive and potentially reflecting natural selection on habitat choices (Martin 1998). Anurans of most species congregate in aquatic habitats during the reproductive season, and male chorus usually exhibit some degree of spatial structuring (Wells 2007), that together with the advertisement call is very importance for the maintenance of species isolation (Hödl 1977, Cardoso et al. 1989, Pombal 1997).

The choice of calling sites may be related to modes of reproduction as well as to morphological, physiological, or behavioral constraints (Crump 1971, Cardoso et al. 1989). In fact, guilds of anuran species use calling sites of different ways, since species whose males call perched on vegetation seems to have more opportunities for segregation than species whose males call on the ground or floating on water surface (Rossa-Feres & Jim 2001, Santos & Rossa-Feres 2007, Vasconcelos & Rossa-Feres 2008). In the last case, differentiation in advertisement calls can explain the species coexistence (Santos & Rossa-Feres 2007). Thus, interspecific differentiation within a water body seems to be a consequence of specific preference for microhabitats (Kopp & Eterovick 2006, Wells 2007). However, assemblage unsaturation (i.e., low species richness per water body) can not be discarded as the reason of the low overlap among anuran species regarding calling sites (Santos & Rossa-Feres 2007, Silva et al. 2008, Vasconcelos & Rossa-Feres 2008).

On the other hand, intraspecific variation in anuran calling sites among water bodies can represent a response to environmental opportunities (i.e., to environmental heterogeneity of water bodies), although this statement was not statistically supported until the present (see examples in Santos & Rossa-Feres 2007 and Vasconcelos & Rossa-Feres 2008).

Historical factors play an important role in the structure of contemporary assemblages (Cadle & Greene 1993). According to Etges (1987), the genetic similarity is expected to be inversely proportional to the overall ecological similarity among species. Indeed, a pattern of ecological changes can occur within anuran species of similar morphologies resulting in resource partitioning (Heyer et al. 1990).

Thus, closely related species would tend to live the same kinds of life but do so in slightly different parts of the habitat so that are spatially separated (Heyer et al. 1990). However, closely related species can also show high ecological similarity, such as reported for lizards (Poe 2005) and snakes (França et al. 2008). The few studies on similarity patterns of calling sites and phylogenetic relatedness in anuran assemblages have reported partial or weak effects of species proximity on microhabitat use (Rossa-Feres & Jim 2001, Eterovick et al. 2008, respectively), highlighting the importance of more studies.

In the last decades, several studies provided important contributions about patterns of anuran calling sites at Brazilian assemblages (Cardoso et al. 1989, Heyer et al. 1990, Haddad & Sazima 1992, Bernarde & Anjos 1999, Bernarde & Kokubum 1999, Bernarde & Machado 2001, Bertoluci & Rodrigues 2002, Toledo et al. 2003, Pombal & Gordo 2004, Conte & Machado 2005, Prado et al. 2005, Zina et al. 2007), but few actually employed statistical approaches to do inferences (Eterovick & Sazima 2000, Rossa-Feres & Jim 2001, Prado & Pombal 2005, Kopp & Eterovick 2006, Santos & Rossa-Feres 2007, Eterovick et al. 2008, Silva et al. 2008, Vasconcelos & Rossa-Feres 2008).

Usually, feasible segregation among anuran species regarding calling sites in previous studies have been assessed performing separated tests for each variable measured (e.g., the height of perching), with no overall tests including several variables in same analysis (e.g., Prado & Pombal 2005, Santos & Rossa-Feres 2007, Vasconcelos & Rossa-Feres 2008), what limit the understanding of general patterns in microspatial distribution of anurans. Another characteristic of previous studies is the temporal schedule employed, which almost always comprise consecutive sampling through several months. Thus, there is a strong temporal component in most datasets that usually have not been considered in analyses (see exceptions in Eterovick & Sazima 2000 and Kopp & Eterevick 2006). Clearly, this feasible temporal variability in calling site use can mask differences or distort patterns of similarity among anuran species if overlooked in analyses or if not minimized in field design.

Herein, we aimed i) to test if anuran species use different calling sites during peaks of breeding activity in temporary ponds; ii) to test if there is relationship between calling site usage and phylogenetic distance among anuran species; iii) to

test if anuran species use different calling sites in the ponds; and iv) to found the subset of calling sites variables that better discriminate anuran species.

## **MATERIAL AND METHODS**

### **Study area and sampling procedure**

We carried out this study at Morro do Diabo State Park (MDSP), a reserve with approximately 33,845 ha located in Teodoro Sampaio municipality, southeastern Brazil (22°27'--22°40'S, 52°10'--52°22'W, 260 to 599.50 m a.s.l.). Vegetation of MDSP is characterized as a mosaic of different stages of regeneration of Mesophytic Semideciduous Forest belonging to the Atlantic Domain (Oliveira-Filho & Fontes 2000, Durigan & Franco 2006) or alternatively to the Misiones Nucleus of the Tropical Seasonal Forests Region (*sensu* Prado 2000). In addition, isolated natural patches of Cerrado *sensu stricto* and a transitional forest ('Myrtaceae Forest') occur within MDSP (Durigan & Franco 2006). Forest composition of Teodoro Sampaio municipality can be explained by great distance of ocean, low annual rainfall, and extensive duration of dry season (Oliveira-Filho & Fontes 2000). Climate is classified as Cwa type of Köppen's (Leite 1998) and historical local records shown annual rainfall ranging from 1,100 to 1,300 mm and indicate mean of temperature of 22°C (Faria 2006).

Temporary ponds fill during rainy seasons (commonly from September to March), and are suitable for the breeding of a high number of anuran species in MDSP (T.G.S. unpubl. data). Pond hydroperiods (i.e., the time in a year when there is standing water at the water bodies, *sensu* van der Valk 2006) varies widely according annual regime of rainfall and individual features of water bodies. Some ponds in MDSP can retain water during whole rainy season, while other fill and dry out two times through one rainy season (e.g., after heavy rainfalls in the onset and in the end of rainy season). During the dry season in MDSP it is usual to occur rains and temporary ponds can fill again.

Here, we studied calling site usage of anuran species in two temporary ponds (TP1 and TP2) located in an alluvial plain of Paranapanema River. These water bodies have very similar characteristics (e.g., low depth, bottom covered by leaf litter and other detritus of terrestrial vegetation, as well as waters with high conductivity, low dissolved oxygen and low richness of potential aquatic predators),

except by canopy cover vegetation (90% in TP1 and <5% in TP2). Bottoms of the monitored temporary ponds present peculiar micro-relief namely 'murundus'; i.e., several small elevations on ground, recovered by grassy and/or arboreal vegetation. Murundus are primarily originate by erosive process and termite activities, and are usually associated to floodplains, wet grasslands, and gallery forests (see references in Resende et al. 2004). The establishment of grassy or herbaceous-arboreal vegetation on murundus is related primarily to size of ground elevations (Resende et al. 2004).

We sampled temporary ponds during short periods, which represented peaks of anuran breeding activity: 22-28 March 2006, 01-07 December 2006, 21-27 Jan 2007, and 01-03 Jan 2009. Therefore, we believe that our sampling schedule minimized temporal variability in the use of calling sites and focused on true spatial measures.

The following variables were recorded for the calling site of each male found:

i) the substratum type: hidden among vegetation on water surface, exposed on water surface, hidden among vegetation on the ground, exposed among vegetation on the ground, perched on herbaceous vegetation, perched on shrub vegetation, and perched on arboreal vegetation;

ii) the pond micro-relief: on edge of the pond, among murundus, at murundus base, and on murundus;

iii) the height above water or ground surface (cm), for species that call from perches;

iv) the water depth (cm), for species that call floating on water;

v) the temporary pond where specimens called: temporary pond in forested area (TP1) and/or opened area (TP2).

### **Statistical analyses**

We tested if males of anuran species used different calling sites performing a one-way Permutational MANOVA test (PERMANOVA) (Anderson 2001a, 2001b). This analysis is a routine for testing the simultaneous response of one or more variables to one or more factors in an ANOVA on basis of any resemblance measure, using permutation methods (Anderson et al. 2008). Thus, the null hypothesis in PERMANOVA is of no differences among groups, with possibility for *a posteriori* pair-

wise comparisons among levels of factors (Anderson et al. 2008) (i.e., to test which anuran species differ significantly from which other species regarding male calling sites).

For PERMANOVA, we calculated an Euclidean Distance resemblance matrix on normalized descriptors of anuran calling sites (*sensu* Anderson et al. 2008). Therefore, we used substratum type and pond micro-relief as categorical predictors (i.e., dummy variables representing multistate categories *sensu* Legendre & Legendre 1998). In addition, we incorporate water depth and perching height as continuous predictors, and we *a priori* designed anuran species as a factor in the analysis. We also constructed a triangular matrix representing phylogenetic distance among anuran species throughout the following numerical categories: (1) species belong to the same species group or clade; (2) species belong to the same genus, but different species group or clade; (3) species belong to the same family, but different genera; (4) species belong to different families. Phylogenetic relationships among anuran species were established according to Heyer (1978), Faivovich et al. (2005), Frost et al. (2006), Nascimento et al. (2006), and Chaparro et al. (2007), summarized in Frost (2009).

In order, we calculated the relationship between phylogenetic distance matrix and calling sites distance matrix by Mantel's test ( $r$ ) (Manly 2000), using 5,000 permutations of Monte Carlo (Smouse et al. 1986).

We employed PERMANOVA test using a crossed unbalanced design to verify if anuran species differed regarding calling sites between the ponds. In this analysis, we included only species with three or more calling sites recorded in each pond, and we considered all variables of calling site measured (except the type of pond, which we included as a second factor in the model).

Finally, we employed General Discriminant Analysis (GDA) to identify which sub-set of calling site predictors better discriminate among anuran species. Traditional Discriminant Analysis (DA) is very similar to analysis of variance (ANOVA/MANOVA) and allows to determine which descriptors (quantitative explanatory variables) discriminate between two or more *a priori* established groups (Legendre & Legendre 1998). Therefore, we used DA to determine the distance among objects (called Mahalanobis Distance) and to test its significance by F-test (Valentin 1995), as well as to calculate the successes of cases classification for each

group. The general proposal of DA is to generate Discriminant Functions that represents the linear combination of original predictors discriminating groups in a multidimensional space (Nelson & Marler 1990). The recent approach of GDA applies the methods of the general linear model to DA problem (StatSoft 2001). Thus, the purpose of GDA is exactly a mix analysis, combining qualitative (categorical) and quantitative (continuous) predictors in the same analysis (StatSoft 2001).

We carried out GDA analysis using 'best' selection, where the adopted procedure was to examine the value of the selection criterion for all possible combinations of predictor variables. Therefore, the program provides the best 1-variable model, the best 2-variable model, and so on. We used significant Wilk's lambda values ( $p < 0.05$ ) as criterion for 'best' selection interpretation, since its standard coefficient ranges from 1.0 (no discriminatory power) to 0.0 (perfect discriminatory power) (StatSoft 2001).

We performed PERMANOVA using PERMANOVA+ add on 1.01 Package (Anderson et al. 2008) in PRIMER-E 6.1.11 software (Clarke & Gorley 2006), and Generalized Discriminant Analysis and Mantel's test in Statistica 6 (StatSoft 2001) and NTSYSpc 2.10s softwares (Rohlf 2000), respectively. For PERMANOVA, we calculated p-values by 9999 Monte Carlo permutations and traditional sequential sums of squares (Type III). We adopted the unrestricted permutation method in one-way tests and permutation of residuals under a reduced model in two-way crossed tests, according Anderson et al. (2008).

## RESULTS

Overall PERMANOVA test rejected the null hypothesis of no differences among vocalization sites of anuran species (pseudo-F = 9.93,  $p < 0.01$ ) and a *posterior* pair-wise comparisons showed that males of most pairs of species (96%) used distinct sites for vocalization activities ( $p < 0.05$ ). Only males of three pairs of species vocalized at undistinguishable sites: *Chiasmocleis albopunctata* and *Physalaemus cuvieri* (pseudo-t = 0.84,  $p = 0.52$ ), *Dendropsophus nanus* and *Scinax similis* (pseudo-t = 1.15,  $p = 0.33$ ), and *Leptodactylus chaquensis* and *Trachycephalus venulosus* (pseudo-t = 1.12,  $p = 0.32$ ). The similarity patterns in calling site use have not relation with phylogenetic distance (Table 1) among anurans

species ( $r = 0.05$ ,  $p = 0.32$ ). The overall intra-specific variability of calling sites ranged from 0 (for *Leptodactylus chaquensis*) to 5.15 (for *Trachycephalus venulosus*) (Table1).

PERMANOVA comparisons revealed that four of six anuran species tested (67%) differed regarding calling sites between the studied ponds (i.e., presented high intra-specific average dissimilarity) (Table2). Indeed, only *Dendropsophus nanus* and *Leptodactylus mystaceus* used similar calling sites in both ponds (Table 2).

The first discriminant function in GDA explained 56% of total dispersion of data, whereas the second discriminant function explained additional 26%, totalizing 82% in the two first functions. In addition, GDA indicated that the best combination of variables discriminating anuran species regarding male calling sites was type of substratum, pond micro-relief, and pond type (Table 3). The type of substratum, pond micro-relief, water depth, and perching height alone also presented discriminatory power (Table 3). Pond type alone and other feasible combinations of predictors were not significant ( $p < 0.05$ ).

The two dimensional ordination of GDA canonical means segregated four guilds of anuran species primarily regarding type of substratum where males called (Figure 1): i) usually on water surface (*Chiasmocleis albopunctata*, *Elachistocleis ovalis*, *Leptodactylus chaquensis*, *Physalaemus cuvieri*, and *Trachycephalus venulosus*), ii) on water surface, but hidden under decaying vegetation (*Leptodactylus podicipinus*), iii) perched on vegetation (*Dendropsophus nanus*, *Scinax fuscovarius*, and *S. similis*), and iv) on the ground (*Leptodactylus fuscus*, *L. mystacinus*, and *L. mystaceus*).

Results of GDA agree to PERMANOVA pair-wise *a posteriori* comparisons, since the same three pairs of anuran species do not differed regarding male calling sites in F-tests for Mahalanobis Distance: *Chiasmocleis albopunctata* and *Physalaemus cuvieri* ( $F = 0.65$ ,  $p = 0.90$ ), *Dendropsophus nanus* and *Scinax similis* ( $F = 1.20$ ,  $p = 0.25$ ), and *Leptodactylus chaquensis* and *Trachycephalus venulosus* ( $F = 0.62$ ,  $p = 0.93$ ). The correct classification of anuran species (i.e., if the model classified the observations in correct groups) was high (76.67%), ranging from 57.14% (*Scinax fuscovarius*) to 100% (*Leptodactylus chaquensis* and *L. fuscus*) (Table 4).

## DISCUSSION

PERMANOVA and GDA analyses had congruent results, showing that most anuran species differed regarding male calling sites. Comparisons with other studies are limited, since tests were usually performed individually for each variable of calling sites instead of overall tests (e.g., Prado & Pombal 2005, Santos & Rossa-Feres 2007, Vasconcelos & Rossa-Feres 2008). Regardless limitations, quantitative analyses in some previous studies reported high species overlap in calling sites (Rossa-Feres & Jim 2001, Kopp & Eterovick 2006, Vasconcelos & Rossa-Feres 2008), while other reported species segregation (Eterovick & Sazima 2000, Santos & Rossa-Feres 2007, Eterovick et al. 2008, Silva et al. 2008). In fact, patterns of microhabitat use can be affected by species composition and/or species richness of local assemblages (Santos & Rossa-Feres 2007, Eterovick et al. 2008). In the present study we recorded that calling site partitioning can occur even in highly opportunistic aggregations of anurans, such as in temporary ponds.

Three pairs of anuran species do not differed regarding male calling sites: *Chiasmocleis albopunctata* and *Physalaemus curvieri*, *Dendropsophus nanus* and *Scinax similis*, and *Leptodactylus chaquensis* and *Trachycephalus venulosus*. Males of *Chiasmocleis albopunctata* and *Physalaemus curvieri* usually called floating on water surface, in murundus base, at temporary pond TP2. However, we observed individuals of the former species calling usually aggregated to patches of vegetation (under dense clumps of dicotyledons), and individuals of *P. curvieri* calling widely dispersed at the pond. Distribution in patches of vegetation was reported for several anuran species in southeastern Brazil (Prado & Pombal 2005). Unfortunately, we do not considered a measure of aggregation in analyses to support our assumptions of calling site segregation between these anuran species.

Males of *Trachycephalus venulosus* showed the high intraspecific variability in calling sites (average distance = 5.15), and no statistical difference compared to *Leptodactylus chaquensis* calling sites. Some males of the former species call perched on arboreal vegetation at temporary pond TP1, but many individuals call floating on water surface, close to *L. chaquensis*, whose males call partially submerged (amidst aquatic vegetation) in deeper portions of the pond.

Males of *Scinax similis* also showed high intraspecific variability in calling sites (average distance = 3.50), since individuals call perched on herbaceous and

arboreal vegetation located inside (on and among murundus) and at the edges of ponds. On the other hand, males of *Dendropsophus nanus* showed lower variability in calling sites (average distance = 2.72), since they call perched only on herbaceous vegetation, usually inside ponds (on and among murundus). The height of perches was very similar between these species (*D. nanus* =  $53.33 \pm 19.34$ ; *S. similis* =  $55.71 \pm 28.68$ ), although the Variation Coefficient was higher for *S. similis* (51.48%) than to *D. nanus* (36.25%). This overlap in perching height seems to be related to limitations in vertical stratification, since most males of *D. nanus* and *S. similis* call at temporary pond TP2, which has vegetation cover comprised predominantly by herbaceous plants. Indeed, environmental heterogeneity was observed allowing differential use of space during calling activities of anurans (Cardoso et al. 1989), and thus anuran calling sites usually show higher overlap in opened habitats probably due to the environmental uniformity (e.g., Cardoso et al. 1989, Haddad & Sazima 1992, Rossa-Feres & Jim 2001, Santos & Rossa-Feres 2007, Vasconcelos & Rossa-Feres 2008).

The high plasticity in calling sites use recorded to hylids can also be explained by the presence of adhesive discs, as well as by the behavioral plasticity of these anuran species, which allow wide utilization of several types of substrate available at water bodies (Cardoso et al. 1989, Pombal 1997, Kopp & Eterovick 2006). Within-group variability (i.e., the intraspecific variability in calling sites) plays an important role in statistical significance of PERMANOVA tests, since large variation can result in non significant p-values (Anderson et al. 2008). Therefore we believe that the great variability in calling sites recorded for males of *Trachycephalus venulosus* and *Scinax similis* explains the no significant differences regarding calling sites of *Leptodactylus chaquensis* and *Dendropsophus nanus*, respectively.

According to GDA analysis, the best sets of calling sites variables discriminating among anuran species in the present study were the combination type of substrate + pond micro-relief + pond type, and substrate type, pond micro-relief, water depth, and perching height alone. Usually, the type of substrate is considered an important variable in most studies of anuran calling sites (e.g., Hödl 1977, Rossa-Feres & Jim 2001, Kopp & Eterovick 2006, Martins et al. 2006, Santos & Rossa-Feres 2007, Eterovick et al. 2008, this study). Here, species guilds of anurans were primarily discriminated by type of substrate, but the interaction of this variable with

pond type and micro-relief clearly explained the pattern of intraguild partitioning for species whose males called from the ground: *Leptodactylus fuscus* called exposed among murundus at pond TP2, whereas *L. mystaceus* called exposed on murundus at pond TP1, and *L. mystacinus* called on murundus, usually hidden among herbaceous vegetation, at ponds TP1 and TP2.

The height of perch has been highlighted as a variable strongly partitioned in calling sites use (e.g., Prado & Pombal 2005, Martins et al. 2006, Kopp & Eterovick 2006, Eterovick et al. 2008), but such tendency have not been corroborated in some studies of water bodies with low stratification of vegetation and consequent few opportunities to partition the vertical dimension (Rossa-Feres & Jim 2001, Santos & Rossa-Feres 2007, this study). In the last case, other variables such as type of substrate and distance from edges can still be partitioned (Rossa-Feres & Jim 2001; Martins et al. 2006, Santos & Rossa-Feres 2007, Eterovick et al. 2008). In fact, in the present study the calling site segregation recorded between *Scinax fuscovarius* and the pair *Dendropsophus nanus* - *Scinax similis* seems more related to type of substrate than height of perch, since males of the former anuran species usually called perched on arboreal vegetation at similar height that the remaining species.

Water depth has not been considered an important variable partitioned among anurans species (Rossa-Feres & Jim 2001, Santos & Rossa-Feres 2007) (but see exception in Eterovick et al. 2008). Therefore, we do not expect to find this variable as important for calling site discrimination. In the present study, water depth together with micro-relief seem to explain the partitioning between calling sites of *Elachistocleis bicolor* and the remaining anuran species whose males called in water surface, since males of the former species called mainly exposed in water surface, among murundus, in shallow portions of the ponds.

The lack of relation between phylogenetic distance and the pattern in calling site use indicates that closely related species do not necessarily show high or low similarity in calling site use. In fact, the pairs of species more similar in calling site use belong to different families, with the exception of *Dendropsopus nanus* and *Scinax similis*. Previous studies reported similar results, since the degree of overlap among distantly related species was equal or even greater than recorded to closely related species (Rossa-Feres & Jim 2001). In addition, the robust data set explored by Eterovick et al. (2008) indicated lack and weak relationship between phylogenetic

relatedness and microhabitat use pattern of tadpoles and adult anurans, respectively. In this last study, the authors argued that this pattern in microhabitat use can not be explained by a simple factor, but rather by combined effects of specific preferences, convergence promoted by environmental conditions, and species interactions.

In fact, calling sites use seems to be very plastic, since we recorded high intraspecific variability for the anuran species between the two studied ponds. This result corroborates the previously reported by Eterovick et al. (2008) and Vasconcelos & Rossa-Feres (2008), but disagree with the reports of Santos & Rossa-Feres (2007), which found that calling sites do not vary among water bodies as a possible response to low environmental heterogeneity (i.e., the vegetation surrounding water bodies was predominantly herbaceous due to anthropogenic pressures). According to Eterovick et al. (2008), the availability of preferred microhabitat types, as well as species interactions and physical properties of each habitat may influence microhabitat choice by anurans. In addition, species with broad niche are good colonizers and adaptable to unpredictable, unstable, or modified habitats (Levins 1968, Heyer & Belin 1973). Clearly, in the present study the ponds TP1 and TP2 presented high structural dissimilarity (TP1 was located inside forest, while TP2 is located in opened area) and great instability/unpredictability due to temporary hydroperiod. Therefore, we considered no surprise that males of anuran species have showed high intraspecific variability in calling sites.

## **Acknowledgements**

This study received financial support from the BIOTA/FAPESP program – The Biodiversity Virtual Institute ([www.biota.org.br](http://www.biota.org.br)). We thank Capes and CNPq for the PhD fellowships given to TGS and TSV, respectively. We are grateful to Morro do Diabo State Park for logistic support, and Marcia Spies for Primer-E use. CFBH thanks FAPESP and CNPq for financial support.

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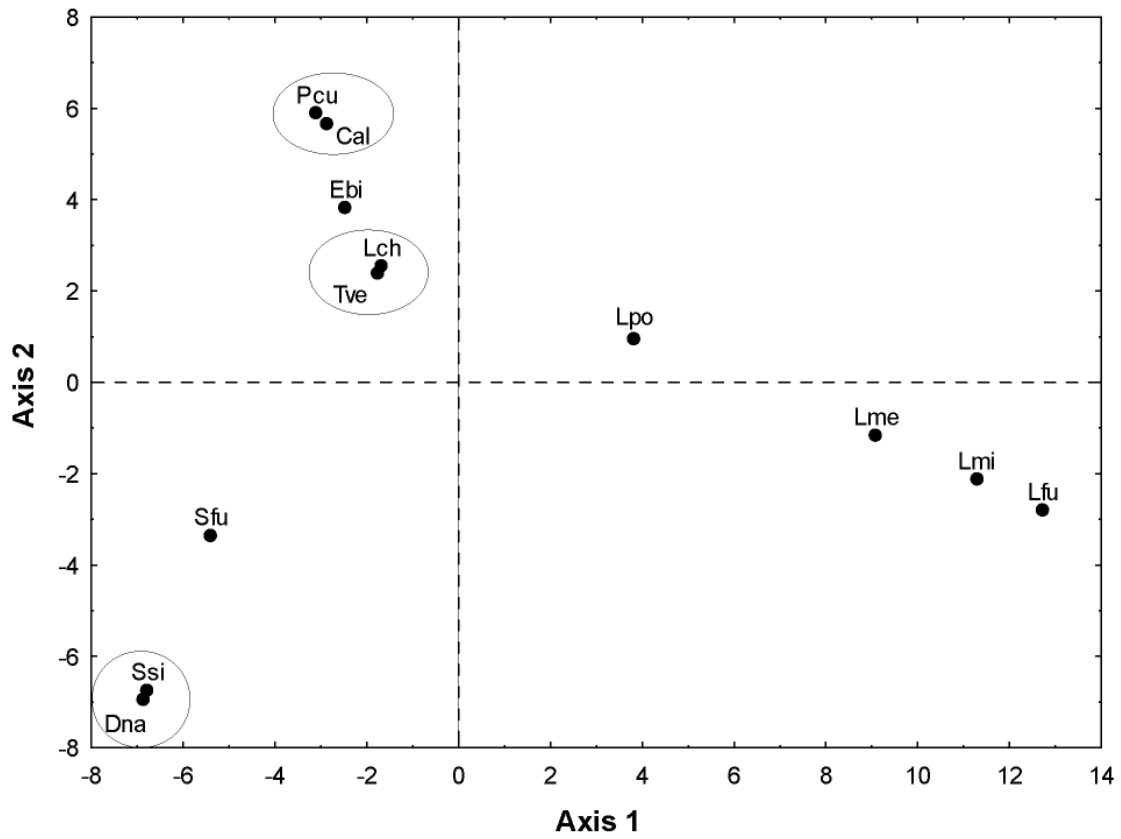
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## FIGURES



**Figure 1.** Micro-distribution of 12 anuran species recorded in temporary ponds, southeastern Brazil, according variables of male calling sites. Axes are roots of Canonical means in General Discriminant Analysis, and circles represent species with no difference in calling sites according to F-test for Mahalanobis Distance. Species abbreviations follow Table1.

## TABLES

**Table 1.** Inter and intra-specific (bold diagonal) average distance of calling sites and phylogenetic distance (italic) of anurans recorded in temporary ponds, southeastern Brazil. Species: *Chiasmocleis albopunctata* (Cal), *Dendropsophus nanus* (Dna), *Elachistocleis bicolor* (Ebi), *Leptodactylus chaquensis* (Lch), *L. fuscus* (Lfu), *L. mystaceus* (Lme), *L. mystacinus* (Lmi), *L. podicipinus* (Lpo), *Physalaemus cuvieri* (Pcu), *Scinax fuscovarius* (Sfu), *S. similis* (Ssi), and *Trachycephalus venulosus* (Tve).

| Species | Cal         | Dna         | Ebi         | Lch      | Lfu         | Lme         | Lmi         | Lpo         | Pcu         | Sfu         | Ssi         | Tve         |
|---------|-------------|-------------|-------------|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Cal     | <b>1.58</b> | 4           | 3           | 4        | 4           | 4           | 4           | 4           | 4           | 4           | 4           | 4           |
| Dna     | 5.24        | <b>2.72</b> | 4           | 4        | 4           | 4           | 4           | 4           | 4           | 3           | 3           | 3           |
| Ebi     | 3.83        | 4.87        | <b>2.63</b> | 4        | 4           | 4           | 4           | 4           | 4           | 4           | 4           | 4           |
| Lch     | 4.48        | 5.63        | 3.47        | <b>0</b> | 2           | 2           | 2           | 2           | 4           | 4           | 4           | 4           |
| Lfu     | 5.55        | 5.48        | 5.39        | 6.21     | <b>4.27</b> | 1           | 1           | 2           | 4           | 4           | 4           | 4           |
| Lme     | 5.92        | 5.48        | 5.48        | 5.94     | 5.24        | <b>3.44</b> | 1           | 1           | 4           | 4           | 4           | 4           |
| Lmi     | 5.74        | 5.30        | 5.43        | 6.04     | 5.46        | 4.76        | <b>3.66</b> | 2           | 4           | 4           | 4           | 4           |
| Lpo     | 5.49        | 5.81        | 4.87        | 5.25     | 6.02        | 5.55        | 6.11        | <b>4.38</b> | 4           | 4           | 4           | 4           |
| Pcu     | 2.12        | 5.31        | 4.11        | 4.86     | 5.54        | 6.02        | 5.82        | 5.73        | <b>2.46</b> | 4           | 4           | 4           |
| Sfu     | 5.76        | 4.24        | 5.52        | 6.11     | 6.30        | 5.47        | 5.48        | 6.20        | 5.88        | <b>3.48</b> | 1           | 3           |
| Ssi     | 5.41        | 3.17        | 5.31        | 6.10     | 5.56        | 5.45        | 5.37        | 6.10        | 5.44        | 4.14        | <b>3.50</b> | 3           |
| Tve     | 5.69        | 5.92        | 4.78        | 3.88     | 6.72        | 6.42        | 6.45        | 5.87        | 5.93        | 5.75        | 6.19        | <b>5.15</b> |

**Table 2.** Pseudo-t tests for intra-specific average distance regarding calling sites of anurans recorded in temporary ponds (TP1 and TP2), southeastern Brazil. Species abbreviations follow Table1.

| Species | Euclidian Distance tests |       |    | Average Distance |      |         |
|---------|--------------------------|-------|----|------------------|------|---------|
|         | Pseudo-t                 | p     | df | TP1              | TP2  | TP1-TP2 |
| Cal     | 2.66                     | 0.02  | 13 | 0.80             | 0.20 | 1.22    |
| Dna     | 1.56                     | 0.11  | 13 | 1.73             | 3.18 | 2.62    |
| Ebi     | 2.36                     | <0.01 | 20 | 3.30             | 0.50 | 2.77    |
| Lmi     | 0.94                     | 0.43  | 18 | 2.54             | 2.87 | 2.63    |
| Sfu     | 9.92                     | <0.01 | 5  | 0.31             | 0.93 | 4.77    |
| Tve     | 2.19                     | 0.01  | 21 | 4.51             | 4.08 | 6.38    |

**Table 3.** Multivariate tests of significance for the best subset of calling site predictors of anurans recorded in temporary ponds, southeastern Brazil.

| Calling site predictors                      | Wilks value | F     | df  | p     |
|----------------------------------------------|-------------|-------|-----|-------|
| Type of substratum * Pond micro-relief *Pond | 0.004       | 4.92  | 176 | <0.01 |
| Type of substratum                           | 0.008       | 24.39 | 44  | <0.01 |
| Pond micro-relief                            | 0.076       | 14.00 | 33  | <0.01 |
| Water depth                                  | 0.434       | 13.28 | 11  | <0.01 |
| Perching height                              | 0.483       | 10.92 | 11  | <0.01 |

**Table 4.** Sample size and correct classifications according to the Discriminant Analysis for calling sites of anurans recorded in temporary ponds, southeastern Brazil. Species abbreviations follow Table1.

| Species      | Sample size | % Correct    |
|--------------|-------------|--------------|
| Cal          | 15          | 80           |
| Dna          | 15          | 66.70        |
| Ebi          | 22          | 81.82        |
| Lch          | 3           | 100          |
| Lfu          | 7           | 100          |
| Lme          | 6           | 83.33        |
| Lmi          | 20          | 80           |
| Lpo          | 9           | 66.67        |
| Pcu          | 9           | 66.67        |
| Sfv          | 7           | 57.14        |
| Ssi          | 14          | 71.43        |
| Tve          | 23          | 78.26        |
| <b>TOTAL</b> | <b>150</b>  | <b>76.67</b> |

## CONSIDERAÇÕES FINAIS

- A anurofauna do Parque Estadual Morro do Diabo (PEMD), o maior remanescente de Floresta Estacional Semidecídua no estado de São Paulo, é composta por 28 espécies de anuros. A anurofauna do PEMD é composta por um misto de espécies atlânticas, do cerrado e amplamente distribuídas na América do Sul, geralmente consideradas tolerantes a modificações antropogênicas.
- A baixa riqueza de espécies e de modos reprodutivos (sete modos), a predominância de espécies habitat-generalistas e a alta similaridade com áreas de Cerrado podem ser explicadas pela sazonalidade climática da área estudada (estação seca pronunciada), além da grande distância em relação a centros de diversificação de anuros, como as montanhas costeiras da Floresta Atlântica úmida.
- A baixa riqueza de espécies registrada e a ausência de endemismos não reduzem a importância do PEMD na manutenção da diversidade de anuros, já que remanescentes florestais podem servir como sítios de abrigo e forrageamento para anuros, bem como de estoques de variabilidade genética.
- A maior riqueza de espécies (considerando machos em atividade de vocalização, ocorrência de girinos e recrutamento de juvenis) ocorreu durante o período de maiores chuvas, temperaturas e fotoperíodos. Entretanto, uma nova proposta de abordagem revelou que apenas a chuva explicou a temporada de vocalização em poças temporárias e apenas o fotoperíodo explicou a temporada em ambientes permanentes (represas e riachos). Assim, enfatizamos a necessidade de uma ampla revisão dos padrões de temporada de vocalização em taxocenoses de anuros neotropicais.
- A análise da distribuição espacial dos anuros também revelou padrões distintos entre os ambientes monitorados, já que três taxocenoses foram identificadas: de riachos permanentes, de represas permanentes e de poças

temporárias. A análise de variáveis ambientais relacionadas aos ambientes aquáticos evidenciou a formação dos mesmos grupos obtidos na análise da anurofauna. Assim, os padrões distribuição espacial registrados sugerem seleção de habitat pelos adultos. Nesse último caso, enfatizamos que a disponibilidade de ambientes aquáticos heterogêneos (tal como corpos d'água lênticos e lóticos, com diferentes hidroperíodos) é um importante mantenedor da diversidade de taxocenoses.

- O estudo da distribuição micro espacial das espécies em duas poças temporárias do PEMD, revelou que os machos da maioria das espécies usaram sítios de vocalização distintos. Desta forma, registramos que segregação nos sítios de vocalização ocorre mesmo em agregações de anuros altamente oportunistas, como aquelas de poças temporárias.
- A combinação de variáveis que melhor discriminou as espécies de anuros em relação aos sítios de vocalização dos machos foi composta por tipo de substrato, micro relevo das poças e tipo de poça. Além disso, o tipo de substrato, micro relevo das poças, profundidade da água e altura dos poleiros isoladamente também apresentaram força discriminatória.
- Os padrões de similaridade no uso dos sítios de vocalização não tiveram relação com a distância filogenética entre as espécies de anuros. Assim, tais padrões podem ser explicados principalmente pela heterogeneidade ambiental das poças estudadas, bem como pela plasticidade ecológica das espécies, já que a maioria das espécies testadas apresentou significativa variabilidade intra específica no uso dos sítios de vocalização entre as poças estudadas.

## Apêndice I



*Rhinella ornata*



*Rhinella schneideri*



*Odontophrynus americanus*



*Dendropsophus minutus*



*Dendropsophus nanus*



*Hysiboas albopunctatus*

Foto: T.S. Vasconcelos



*Hypsiboas faber*



*Hypsiboas punctatus*



*Hypsiboas raniceps*



*Itapotihyla langsdorffii*



*Pseudis platensis*



*Scinax berthae*

Foto: T.S. Vasconcelos

Foto: T.S. Vasconcelos



*Scinax fuscomarginatus*



*Scinax fuscovarius*



*Scinax similis*



*Trachycephalus venulosus*



*Leptodactylus chaquensis*



*Leptodactylus fuscus*



*Leptodactylus mystaceus*



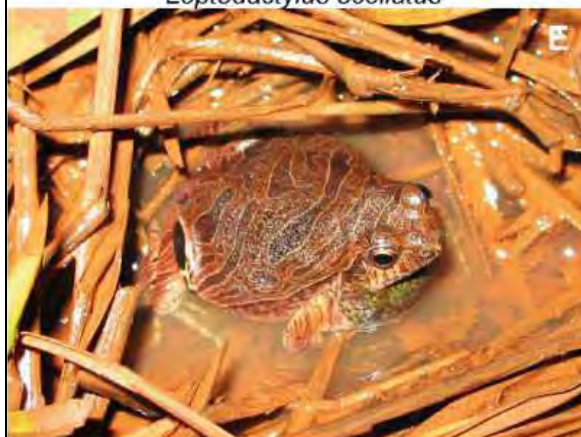
*Leptodactylus mystacinus*



*Leptodactylus ocellatus*



*Leptodactylus podicipinus*



*Eupemphix nattereri*



*Physalaemus cuvieri*



*Chiasmocleis albopunctata*



*Elachistocleis bicolor*

## Apêndice II



Riacho permanente - Córrego do Caldeirão



Riacho permanente - Córrego da Onça



Represa permanente



Represa permanente



Poça temporária



Poça temporária