UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE ANIMAL

ECOLOGIA COMPORTAMENTAL DE *CROSSODACTYLUS SCHMIDTI* GALLARDO, 1961 (ANURA, HYLODIDAE): ATIVIDADE REPRODUTIVA, COMUNICAÇÃO ACÚSTICA, VISUAL E MULTIMODAL

TESE DE DOUTORADO

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Santa Maria, RS, Brasil

2015

PPGBA/UFSM, RS

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Doutor

2015

ECOLOGIA COMPORTAMENTAL DE *CROSSODACTYLUS SCHMIDTI* GALLARDO, 1961 (ANURA, HYLODIDAE): ATIVIDADE REPRODUTIVA, COMUNICAÇÃO ACÚSTICA, VISUAL E MULTIMODAL

Vinícius Matheus Caldart

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

Orientadora: Prof. Dra. Sonia Zanini Cechin

Santa Maria, RS, Brasil

2015

Ficha catalográfica elaborada através do Programa de Geração Automática
da Biblioteca Central da UFSM, com os dados fornecidos pelo(a) autor(a).

Caldart, Vinícius Matheus
Ecologia comportamental de Crossodactylus schmidti Gallardo, 1961 (Anura, Hylodidae): atividade reprodutiva, comunicação acústica, visual e multimodal / Vinícius Matheus Caldart.-2015.
182 p.; 30cm

Orientadora: Sonia Zanini Cechin
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Biodiversidade Animal, RS, 2015

1. Comunicação 2. Reprodução 3. Fatores ambientais 4. Razão sinal-ruído 5. Receptores ilegítimos I. Cechin, Sonia Zanini II. Título.

Universidade Federal de Santa Maria
Centro de Ciências Naturais e Exatas
Programa de Pós-Graduação em Biodiversidade Animal

A Comissão Examinadora, abaixo assinada,
aprova a Tese de Doutorado

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elaborada por
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como requisito parcial para obtenção do grau de **Doutor em Ciências Biológicas – Área Biodiversidade Animal**

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Santa Maria, 26 de fevereiro de 2015.

AGRADECIMENTOS

A realização desta tese só foi possível devido à colaboração e apoio de muitas pessoas e instituições que, de diferentes formas, contribuíram para o desenvolvimento da minha maturidade científica e profissional. Desde 2009 passei a participar do **PPG Biodiversidade Animal** e do **Laboratório de Herpetologia da UFSM**, o que me permitiu trocar conhecimentos com professores, colegas e amigos da graduação, do mestrado e do doutorado no dia-a-dia das disciplinas, em campo ou em conversas discontraídas nos corredores e em churrascos de final de semana. Foi um período muito frutífero e proveitoso da minha formação acadêmica, e o convívio com essas pessoas fará com que eu lembre sempre com carinho da minha estadia em Santa Maria.

Agradeço a todos os colegas do **Laboratório de Herpetologia**, por terem me ajudado ao longo desses quatro anos com sugestões, incentivos e críticas pertinentes, o que tornava o nosso convívio muito agradável. À minha orientadora, **Dra. Sonia Zanini Cechin**, pela confiança e interesse no meu trabalho e por ter contribuído para que o doutorado acontecesse, ajudando com a logística e obtenção de recursos para a coleta de dados. Ao **Victor Lipinski**, **Bruno Madalozzo**, **Maurício Beux dos Santos**, **Marcelo C. da Rocha**, **George Polidoro** e **Samanta Iop**, por terem ajudado no trabalho pesado de campo (sem reclamar por ter que acordar mais cedo que um ornitólogo), e à **Luiza Loebens**, **Amanda Brum** e **Lívia Bataioli** pela inestimável ajuda em laboratório. Ainda, pelas conversas que contribuíram muito para meu crescimento ao longo do doutorado, agradeço aos colegas **Victor Lipinski**, **Bruno Madalozzo**, **Maurício Beux dos Santos, Tiago Bertaso, Tiago Theis, Paulo Mesquita**, **Daniel Cassiano Lima**, **Franciele Maragno**, **Carolina Pietczak**, **Ruben Bolter**, **Jonas Sponchiado, Geruza Melo** e **Franchesco Della Flora.** Ao secretário do PPGBA, **Sidnei Cruz**, por resolver muitos dos problemas burocráticos da vida de um pós-graduando.

Agradeço à **Doris Preininger**, **Thomas Grafe**, **Art Borkent**, **Glauco Machado**, **Rodrigo Lingnau**, **Luis Giasson**, **Camila Both, Cynthia Prado, Selvino Neckel de Oliveira, Sandro Santos**, **Luciane Ayres Peres**, **Vanessa Fortes**, **Tiago Gomes dos Santos** e **Fabio Perin de Sá**, por terem dedicado parte de seu tempo para avaliar meus manuscritos e/ou para me ajudar a sanar dúvidas referentes a análises e conteúdos específicos. A **CAPES** – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, pela bolsa de doutorado concedida. A **SEMA-RS** – Secretaria de Estado de Meio Ambiente do Rio Grande do Sul, pela permissão para realizar a pesquisa no Parque Estadual do Turvo, e ao **ICMBio/IBAMA** – Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, pela concessão da licença de coleta. Ao gestor do Parque Estadual do Turvo, **Dante Meller**, e aos guardas-parque da unidade pelas informações e ajuda logística prestada ao longo do estudo. Ao **Enio Elsenbach e família,** pelas conversas e gentilezas, e por me permitirem adentrar sua propriedade para chegar ao riacho Falcão quase que todo mês. Não me esquecerei das jantas e daquela vez que levaram uma coca-cola gelada para mim no riacho! Às **rãzinhas das pedras** do Parque Estadual do Turvo, que já não tem mais muita privacidade devido a minha intromissão constante em suas vidas.

Agradeço aos amigos **Victor Lipinski**, **Tiago Bertaso**, **Bruno Madalozzo**, **Carla Hendges**, **Maurício Beux dos Santos, Marcelo C. da Rocha**, **Jonas Sponchiado**, **Geruza Melo**, **Pablo Paim**, **Marcelo Dalosto** e **Franchesco Della Flora,** pela parceria, amizade, apoio e suporte nos momentos que precisei. Agradeço de coração aos meus pais, **Vitalino Caldart** e **Roselei Caldart**, e a minha irmã **Fabiola Caldart**, pelo carinho, confiança, apoio e suporte que só a família pode dar! Nos momentos de desânimo e cansaço a motivação e confiança de vocês foram fundamentais! Ao **Gilmar**, **Giuliana**, **Andressa** e **Eloiza Iop**, pelo apoio constante e pela confiança em mim. Muito obrigado!

E, é claro, não poderia faltar o agradecimento à pessoa que mais contribuiu para as alegrias da minha vida pessoal e profissional: minha noiva, bióloga, colega de doutorado, de laboratório, de profissão, colaboradora nas pesquisas e nas peripécias da vida, **Samanta Iop**. Se realizar um doutorado é um desafio enorme, imagine como é se sentir como se estivesse fazendo dois doutorados... Sim, por que nós nos ajudamos mutuamente ao longo destes quatro anos! Obrigado pela ajuda em campo, em laboratório, em casa, interpretando e discutindo os resultados, opinando em cada figura e tabela, aguentando minha empolgação ou frustração diante dos acontecimentos. Obrigado pela confiança, pelo respeito, amor e carinho que sente por mim, por estar sempre disposta a sorrir e por dar o apoio necessário para a conclusão deste trabalho. Amo você!

Meu sincero agradecimento **a todas as pessoas mencionadas**, pois sem a ajuda de vocês as dificuldades ao longo desse desafio teriam sido muito maiores. Obrigado!

“Somewhere, something incredible is waiting to be known”

Carl Sagan

RESUMO

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

ECOLOGIA COMPORTAMENTAL DE *CROSSODACTYLUS SCHMIDTI* GALLARDO, 1961 (ANURA, HYLODIDAE): ATIVIDADE REPRODUTIVA, COMUNICAÇÃO ACÚSTICA, VISUAL E MULTIMODAL

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Data e Local de Defesa: Santa Maria, 26 de fevereiro de 2015.

O processo de comunicação possui papel crucial na história de vida dos organismos, pois assume uma interação coevolutiva entre emissor e receptor. A comunicação acústica é um aspecto fundamental da história de vida dos anuros, visto que é utilizada para mediar interações sociais, influencia nos riscos de predação e parasitismo e está diretamente ligada a reprodução e sucesso reprodutivo. Entretanto, espécies que habitam ambientes acusticamente complexos, como riachos, enfrentam um problema ecológico inerente desse tipo de hábitat, que interfere na comunicação acústica: o ruído das corredeiras. Notavelmente, a comunicação visual evoluiu de modo convergente em diversas famílias de anuros diurnos de riacho e, além disso, algumas dessas espécies também utilizam sinais visuais e acústicos associados em displays multimodais. Eu investiguei a distribuição geográfica, história natural, ecologia e comportamento da rã diurna de riacho *Crossodactylus schmidti* (Hylodidae) no sul do Brasil, abordando questões relativas à atividade reprodutiva e comunicação através de observações e experimentos *in situ* e por meio da análise de indivíduos coletados. Acerca da atividade reprodutiva, avaliei os padrões temporais da atividade de canto dos machos e o efeito das variáveis ambientais sobre a atividade diária/mensal, os padrões temporais de maturação sexual, as relações "tamanho x fecundidade" e o investimento reprodutivo de machos e fêmeas. Acerca da comunicação, investiguei as pressões seletivas que influenciam na estrutura dos sinais (*adaptações*) e no uso de estratégias na comunicação (*plasticidade*), descrevi o repertório acústico, visual e multimodal, o uso do microhábitat para emissão dos sinais visuais/multimodais, e avaliei relações "sinal acústico x ruído ambiente" e a interação entre mosquitos parasitas e machos de anuros. A comunicação em *C. schmidti* é complexa e envolve um amplo repertório de sinais acústicos, visuais e multimodais. Machos possuem atividade de canto contínua ao longo do ano, com efeitos variáveis dos fatores ambientais: a chuva acumulada e a temperatura do ar afetaram a atividade mensal de canto, enquanto a intensidade luminosa e a temperatura do ar afetaram a atividade diária. O padrão de atividade reprodutiva de *C. schmidti* no sul do Brasil é do tipo contínuo, com a presença de indivíduos sexualmente maduros de ambos os sexos ao longo do ano, o que sugere forte competição intrasexual e que o sistema de acasalamento é do tipo "poliginia por defesa de recursos". Parte das notas que compõe o canto trinado apresentou razão sinal-ruído negativa nos cantos de todos os machos analisados. Diante do efeito do mascaramento do ruído ambiente sobre a transmissão do sinal acústico, *C. schmidti* apresenta adaptações (e.g., canto trinado, comunicação visual), bem como plasticidade em traços acústicos (e.g., modulação de frequência dominante) relacionada às mudanças súbitas/temporárias no ruído ambiente. Os mosquitos do gênero *Corethrella* foram de fato atraídos por cantos de anuros, enquanto mosquitos dos gêneros *Forcipomyia* e *Uranotaenia* não; este padrão foi reforçado pela ausência de diferença no tratamento com um ruído branco e armadilhas silenciosas adjacentes. Contudo, o padrão de abundância de *Corethrella* é baixo em relação a áreas tropicais. Estudar uma espécie singular como *C. schmidti* fornece uma valiosa oportunidade para o entendimento da evolução de estratégias temporais de reprodução e de adaptações e estratégias de comunicação em anuros diurnos de riachos.

Palavras-chave: Sinais. Ambiente de sinalização. Atividade de canto. Fatores ambientais. Razão sinal-ruído. Adaptação acústica. Plasticidade acústica. Reprodução. Receptores ilegítimos.

ABSTRACT

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

BEHAVIORAL ECOLOGY OF *CROSSODACTYLUS SCHMIDTI* GALLARDO 1961 (ANURA, HYLODIDAE): REPRODUCTIVE ACTIVITY, ACOUSTIC, VISUAL AND MULTIMODAL COMMUNICATION

AUTHOR: VINÍCIUS MATHEUS CALDART
ADVISOR: SONIA ZANINI CECHIN

The communication process has a fundamental role in the life history of organisms, as it assumes a coevolutionary interaction between sender and receiver. The acoustic communication has a key role in the life history of anurans, as it is used to mediate social interactions, influences the risk of predation and parasitism and is directly linked to reproduction and reproductive success. However, species that inhabit acoustically complex environments, such as streams, face an ecological problem inherent of this type of habitat that affects acoustic communication: the noise produced by stream torrents. Remarkably, the visual communication has convergently evolved in several families of diurnal stream frogs and, in addition, some of these species are known to also use visual and acoustic signals associated within multimodal displays. I investigated the geographic distribution, natural history, ecology and behavior of the diurnal stream frog *Crossodactylus schmidti* (Hylodidae) in southern Brazil, addressing questions relative to the reproductive activity and communication through *in situ* observations and experiments and through the analysis of collected specimens. Regarding the reproductive activity, I evaluated the temporal patterns of male calling activity and the effect of environmental factors on daily/monthly activity, the temporal patterns of sexual maturity, the "size x fecundity" relationships and the reproductive investment of males and females. Regarding communication, I investigated the selective pressures that influence the structure of signals (*adaptations*) and the use of strategies in communication (*plasticity*), described the acoustic, visual and multimodal repertoire, the microhabitat used to emission of visual/multimodal displays, and investigated relations between "acoustic signal x ambient noise" and the interaction between parasitic flies and calling male frogs. Communication in *C. schmidti* is complex and involves a wide repertoire of acoustic, visual and multimodal signals. Males have continuous calling activity throughout the year, with varying effects of environmental factors: the accumulated precipitation and air temperature affected the monthly calling activity, while the light intensity and air temperature affected the daily activity. The pattern of reproductive activity of *C. schmidti* in southern Brazil fits into the continuous type, with the presence of sexually mature individuals of both sexes throughout the year, which suggests strong intrasexual competition and that the species' mating system is a "resource defense polygyny". Some of the notes that compose the species multi-note advertisement call showed negative signal-to-noise ratio for calls of all analyzed males. Given the masking effect of the ambient noise on the acoustic signal transmission, *C. schmidti* presents acoustic adaptations (e.g., multi-note call, visual communication), and plasticity in acoustic traits (e.g., dominant frequency modulation) related to sudden/temporary changes in ambient noise. Flies of the genera *Corethrella* were indeed attracted to frog calls, while *Forcipomyia* and *Uranotaenia* flies were not; this pattern was reinforced by the lack of difference in the treatment with a white noise and adjacent silent traps. However, the pattern of abundance of *Corethrella* is much lower when compared to tropical areas. Studying a singular species as *C. schmidti* provides a valuable opportunity to understand the evolution of temporal strategies of reproduction, adaptations and behavioral plasticity related to the communication of diurnal stream frogs.

Keywords: Signals. Signaling environment. Calling activity. Environmental factors. Signal-to-noise ratio. Acoustic adaptation. Acoustic plasticity. Reproduction. Eavesdroppers.

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1. INTRODUÇÃO

1.1. Uma investigação interdisciplinar do comportamento

Esta tese é composta por estudos de natureza descritiva, estudos que incluem testes de hipóteses biológicas e estudos que utilizam ambas as abordagens, ou seja, que descrevem padrões e que testam relações entre variáveis. O conteúdo apresentado nesta tese engloba conhecimentos que podem ser interpretados sob as perspectivas de pelo menos três importantes disciplinas biológicas: a *História Natural*, a *Etologia* e a *Ecologia Comportamental*. Embora variem bastante em suas abordagens conceituais e metodológicas, estas disciplinas são sem dúvida complementares e de aplicação fundamental na investigação de questões biológicas centradas no paradigma evolutivo.

Pode-se argumentar que há um avanço temporal nessas disciplinas, tanto teórico quanto metodológico, que vai desde a História Natural clássica, que visa descrever como vivem os organismos, passando pela Etologia, que descreve os padrões fixos de ação (i.e., os comportamentos) e os interpreta como traços fenotípicos que possuem uma história evolutiva, indo até a Ecologia Comportamental, que analisa como os organismos lidam com problemas ecológicos diversos através dos comportamentos, adaptações e plasticidade, e como a variação individual afeta a aptidão reprodutiva. De fato, se considerarmos um panorama amplo no estudo do comportamento animal, os conhecimentos destas três disciplinas se fundem e se tornam complementares. Entretanto, cada uma delas possui fundamentações teóricas e conceituais que as caracterizam individualmente. Nesta seção, apresento brevemente as relações entre estas disciplinas os tópicos que compõe esta tese.

Todos os organismos possuem uma "história natural" moldada por processos evolutivos, que ao longo de muitas gerações ocasionaram mudanças em diferentes níveis fenotípicos, como, por exemplo, em sequências genotípicas, em traços morfológicos e em traços comportamentais (RIDLEY, 2006). Assim, a "história natural" enquanto disciplina pode ser entendida como a observação e descrição do mundo natural, com o estudo dos organismos e suas relações com o ambiente tendo importância central (TEWKSBURY et al., 2014), enquanto que o estudo da "história de vida" dos organismos pode ser entendido como a observação e descrição das adaptações às pressões evolutivas sob as quais os organismos estiveram historicamente expostos (e.g., "long-term adaptations" *sensu* BRUMM; SLABBEKOORN, 2005), e também das respostas imediatas que surgem através da plasticidade fenotípica (e.g., "short-term adaptations" *sensu* BRUMM; SLABBEKOORN, 2005). O estudo da evolução das diferentes histórias de vida é, em suma, uma busca por padrões e por explicações para esses padrões (BEGON et al., 2007).

O termo “história de vida” é, contudo, bastante amplo e engloba uma variedade de tópicos. A história de vida de um organismo compreende, durante seu ciclo de vida, desde o padrão de crescimento, desenvolvimento, a armazenagem de recursos para suas atividades, até a reprodução, senescência e morte (ROFF, 1992; BEGON et al., 2007). Estudos de história natural, em geral, abrangem diversos aspectos entendidos como essenciais na história de vida dos organismos, como a reprodução, ecologia alimentar, relações intraespecíficas e interespecíficas, e relações dos organismos com as variáveis abióticas do ambiente (STEBBINS; COHEN, 1995; GIRAUDO, 2002). Tais estudos fornecem informações biológicas fundamentais que, muito frequentemente, constituem o conhecimento basal para a investigação de questões específicas advindas de linhas de pesquisas de outras disciplinas. Para exemplificar, muito do conhecimento sobre ecologia e comportamento de anfíbios adveio de estudos de história natural que buscaram descrever padrões temporais e comportamentais de atividade de vocalização e reprodução (e.g., CRUMP, 1974; WELLS, 1977), padrões de uso dos recursos alimentares (e.g., TOFT, 1980, 1981), padrões de maturação das células sexuais (e.g., BURGOS; FAWCETT 1956), entre outros. Atestando a importância de investigar a história natural dos organismos, estudos recentes reuniram informações de história natural de anfíbios anuros e forneceram importantes contribuições sobre a evolução e a generalidade de padrões comportamentais relacionados ao uso de sinais visuais na comunicação (e.g., HÖDL; AMÉZQUITA, 2001; HARTMANN et al., 2005), estratégias e modos reprodutivos (HADDAD; PRADO, 2005), estratégias e mecanismos defensivos (TOLEDO; HADDAD, 2009; TOLEDO et al., 2011) e dimorfismo sexual em tamanho (NALI et al., 2014). Portanto, a história natural que fora fundamentada nos séculos XVIII e XIX continua subsidiando conhecimentos essenciais a outras disciplinas (BURY, 2006; TEWKSBURY et al., 2014) e, assim, ganhando um caráter cada vez mais interdisciplinar na ciência (TEWKSBURY et al., 2014).

A Etologia, por sua vez, ganhou a proeminência de uma disciplina científica nas décadas de 1940 e 1950, a partir dos fundamentos teóricos propostos por Konrad Lorenz, Nikolaas Tinbergen e Karl Von Frisch, e em completa oposição a escola americana da psicologia comparada, que entendia à época que todo comportamento era fruto de aprendizado. Tinbergen argumentou que qualquer traço morfológico ou comportamental de um organismo poderia ser investigado a partir de quatro questões ou níveis independentes. Estes dizem respeito a *1) estrutura ou mecanismo*, a *2) ontogenia*, a *3) função* e a *4) filogenia* do traço fenotípico. As duas primeiras são entendidas como *"questões proximais"*, pois se referem aos determinantes internos (do organismo) e externos (do ambiente) que regulam o comportamento (*estrutura ou mecanismo*), e a como o comportamento se desenvolve ao longo da vida do indivíduo (*ontogenia*). As duas últimas questões são chamadas de *"questões finais"*, no sentido de que procuram investigar os determinantes evolutivos que atribuem valor adaptativo de determinado comportamento ao organismo (*função*), e a história evolutiva de determinado comportamento em espécies relacionadas (*filogenia*) (NOBLE, 1998; YAMAMOTO, 2006). Assim, na perspectiva etológica temos quatro níveis para a análise de um traço fenotípico qualquer, que são independentes entre si no sentido de que a análise de um nível não se opõe a análise de outro. Na verdade, a investigação de cada nível contribui com elementos que permitem integrar o conhecimento de pesquisas em outros níveis de análise, integrando com o tempo as descobertas em uma explicação mais satisfatória (ALCOCK, 2009).

A partir das quatro questões formuladas por Tinbergen, a investigação dos processos evolutivos e das pressões ecológicas que moldam os comportamentos deu origem a Ecologia Comportamental. Do ponto de vista da Ecologia Comportamental, a premissa máxima é de que os organismos estão historicamente expostos a "problemas ecológicos", de modo que os comportamentos devem evoluir para maximizar a aptidão dos indivíduos que possuem traços comportamentais adaptativos em relação às pressões ecológicas (OWENS, 2006). Assim, o conceito de adaptação possui papel central no panorama conceitual da Ecologia Comportamental, e aspectos como a variação individual nos traços comportamentais e as consequências adaptativas dessas variações são importantes princípios dessa disciplina (KREBS; DAVIES, 1996). Uma abordagem que contribuiu bastante para o entendimento da evolução de adaptações na história de vida dos organismos é a da "otimização", que estabelece que a combinação observada dos componentes da história de vida de um dado organismo deve ser aquela que fornece um maior valor adaptativo para o organismo na natureza (STEARNS, 2000; WELLS, 2007). Dentre as abordagens alternativas à da otimização, por exemplo, está a que estabelece que a aptidão individual também pode ser "dependente da frequência", quando a seleção para uma determinada característica varia de acordo com a densidade populacional (SINERVO et al., 2000; BEGON et al., 2007).

Por estarem diretamente relacionadas à aptidão individual, a comunicação e a reprodução são aspectos fundamentais da história de vida de muitos organismos, e a investigação de padrões de reprodução e de comunicação fornecem um rico material sobre a adaptação destes traços às condições locais. Estudos sobre comunicação e reprodução de anfíbios anuros são abundantes na literatura de história natural, mas menos abundantes na literatura especializada em etologia e ecologia comportamental. OWENS (2006) demonstrou que houve um aumento nos estudos sobre seleção sexual e comunicação animal no período entre 1980-2004, e que entre 2001-2005 aproximadamente 90% dos estudos publicados em periódicos de ecologia comportamental utilizaram aves, insetos, peixes ou mamíferos como organismos modelos, com cerca de 50% utilizando uma abordagem observacional. O ensaio de Owens não apresenta dados definitivos, mas chama a atenção para a ausência de herpetólogos adentrando no campo de ecologia comportamental, ou, alternativamente, que há certa resistência por parte dos ecólogos comportamentais em utilizar anfíbios como organismos modelos. De maneira geral, estudos com anfíbios centrados nos referenciais teóricos da ecologia comportamental têm aumentado no Brasil apenas na última década (e.g., CAMPOS et al., 2014), enquanto outros países possuem tradição nessa área há mais tempo (RYAN, 1980; TUTTLE; RYAN, 1981; GRAFE, 1996). Dentre os fatores que influenciam esse quadro pode estar o fato de que a ecologia comportamental é uma disciplina recente, de que há poucos pesquisadores com formação em ecologia comportamental no Brasil, e porque dados básicos de história natural são desconhecidos para a maioria das nossas espécies, dificultando o delineamento de estudos de testes de hipóteses com organismos modelos adequados.

Na presente tese, em relação à comunicação (ver itens 1.2. e 1.4.), investiguei principalmente questões relacionadas à estrutura dos sinais e aos processos seletivos que podem ter moldado as adaptações na comunicação. Investiguei em especial as pressões seletivas que influenciam na estrutura dos sinais (*adaptações*) e no uso de estratégias na comunicação (*plasticidade*), descrevendo o repertório acústico, visual e multimodal, testando hipóteses acerca do uso do microhábitat para emissão dos displays visuais e multimodais, avaliando as relações “sinal acústico x ruído ambiente”, e investigando a potencial pressão seletiva de mosquitos parasitas orientados por fonotaxia ao macho anuro hospedeiro. Por fim, em relação à atividade reprodutiva (ver itens 1.2. e 1.4.), investiguei os padrões temporais de desenvolvimento e maturação das células sexuais de machos e fêmeas, as relações “tamanho x fecundidade” e o investimento reprodutivo, a ocorrência de indivíduos maduros ao longo do ano e a variação diária e mensal na atividade de vocalização, testando hipóteses acerca dos efeitos das variáveis ambientais sobre a atividade de canto dos machos. Assim, nos capítulos que compõe esta tese estão presentes abordagens conceituais e teóricas advindas da história natural, da etologia e da ecologia comportamental, as quais são abordadas com maior profundidade em cada capítulo.

1.2. Comunicação e reprodução em anfíbios anuros

Os sinais são os blocos básicos da comunicação e evoluem a partir da interação coevolutiva entre emissor e receptor (HEBETS; PAPAJ, 2005). Através das informações transmitidas pelos sinais, um indivíduo pode avaliar atributos de outro em interações intra e interespecíficas, e a associação entre sinais e respostas constitui o processo de comunicação (SCOTT-PHILLIPS et al., 2012). Em anfíbios anuros a comunicação acústica é considerada uma característica ancestral, pois a maioria das espécies possui hábito noturno e o sinal acústico não é dependente de luminosidade (WELLS, 2007). Além disso, a comunicação acústica tem papel central na história de vida dos anuros, pois media as interações sociais (WELLS, 1977), influencia nos riscos de predação e parasitismo (TUTTLE; RYAN, 1981; GRAFE et al., 2008) e está diretamente ligada ao sucesso reprodutivo (RYAN, 1980; GERHARDT, 1991). O repertório acústico varia bastante entre as espécies, mas geralmente os anuros apresentam pelo menos um canto reprodutivo (canto de anúncio) e um canto agressivo (e.g. territorial, agonístico) (WELLS, 2007). Por ser espécie-específico e estar diretamente relacionado à reprodução, o canto de anúncio constitui um mecanismo de isolamento reprodutivo e é moldado por pressões de seleção natural e sexual (BLAIR, 1958).

A partir de um sistema geral de comunicação acústica em anuros (Figura 1), destacam-se quatro questões: *1)* as interações coevolutivas entre emissor e receptor, e.g. fêmea co-específica; *2)* as interações coevolutivas entre emissor e audiência não-alvo, e.g. machos co-específicos, predadores, parasitas; *3)* a influência das características físicas do ambiente na transmissão do sinal, e.g. densidade da vegetação, ruído ambiente; e *4)* a interpretação do sinal e resposta do receptor. Tais questões referem-se basicamente a *i)* estrutura e *ii)* função do sinal, e *iii)* às pressões seletivas que afetam sua estrutura e função, de modo que podem ser investigadas independentemente. Enquanto o sinal é transmitido em direção ao receptor alvo, receptores ilegítimos como predadores, parasitas, parasitoides ou machos co-específicos podem explorá-lo em seu benefício (BERNAL et al., 2006; ZUK et al., 2006; HALFWERK et al., 2014). Além disso, as características físicas do habitat, como a densidade de vegetação e o ruído biótico e/ou abiótico do ambiente, interferem na maneira como o sinal chega ao receptor alvo e é interpretado por este, influenciando assim a sua reposta. Deste modo, da perspectiva do emissor é esperado que ocorra a evolução de adaptações e o uso de estratégias comunicativas para lidar com o problema do ruído ambiente (BRUMM; SLABBEKOORN, 2005) e da pressão seletiva dos receptores ilegítimos sobre a comunicação (ZUK; KOLLURU, 1998; PEAKE, 2005).

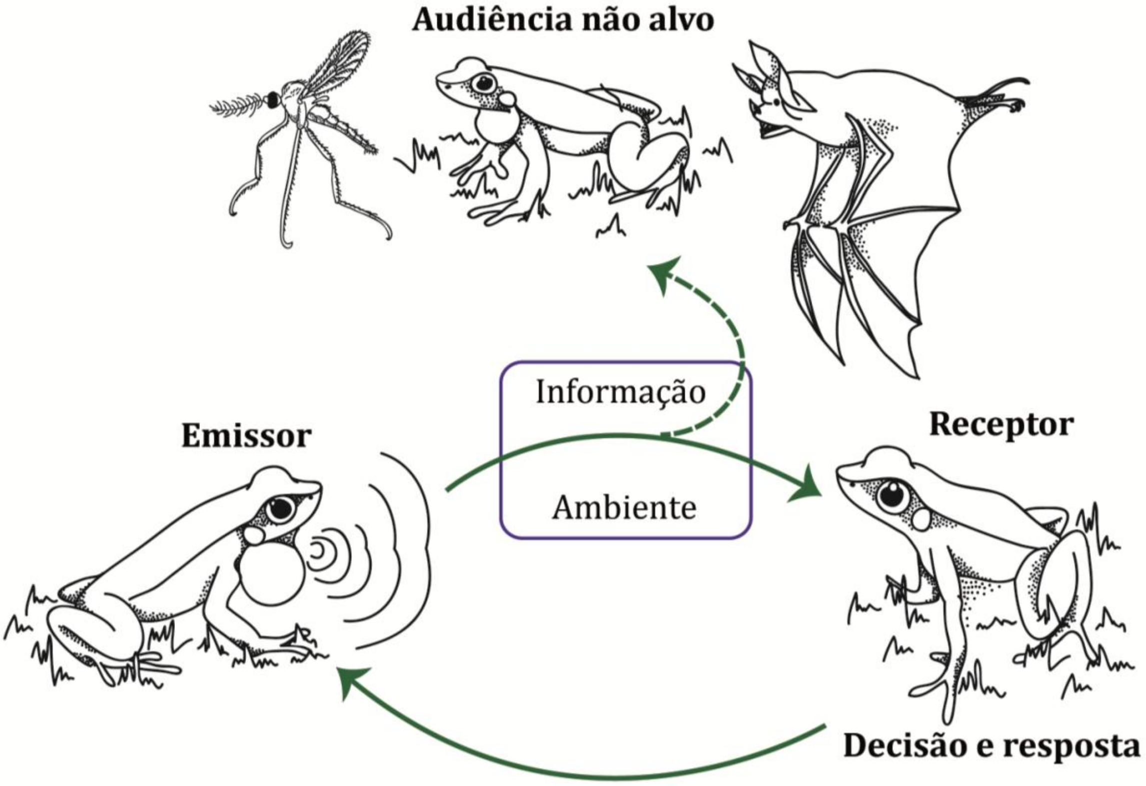

Figura 1. Modelo geral de um sistema de comunicação acústica em anfíbios anuros, destacando a interação coevolutiva entre o emissor e o receptor legítimo, e entre o emissor e receptores ilegítimos. O sinal acústico do indivíduo emissor é direcionado ao indivíduo receptor alvo, mas pode ser interceptado por receptores ilegítimos que, em seu benefício, podem explorar o sinal, exercendo pressões seletivas variáveis sobre o emissor. A audiência não alvo de um sinal acústico pode incluir indivíduos co-específicos, predadores e parasitas ou parasitoides. Enquanto o sinal acústico é transmitido, suas propriedades podem sofrer influência das características físicas do hábitat, como a densidade da vegetação, o ruído ambiente e as condições atmosféricas. O grau de influência das propriedades físicas do hábitat sobre o sinal pode afetar a forma como o sinal chega ao receptor e é interpretado por este, influenciando assim sua resposta. Ilustração: Andressa Iop.

Dado que os anfíbios anuros constituem um grupo de animais acusticamente ativos, o ruído ambiente constitui uma importante pressão seletiva que influência na evolução de adaptações e estratégias de comunicação. Para lidar com o ruído ambiente, muitas espécies apresentam adaptações ou estratégias que permitem aumentar a razão sinal-ruído, aumentando assim a probabilidade do sinal emitido ser corretamente recebido e interpretado pelo receptor (ENDLER, 1992, 1993). Dentre as adaptações estão cantos com altas frequências dominantes (HADDAD; GIARETTA, 1999; BRUMM; SLABBEKOORN, 2005) ou mesmo frequências ultrasônicas (ARCH et al., 2008), e o uso de modalidades adicionais de comunicação, como a transmissão de sinais através de movimentos dos membros ou posturas corpóreas (LINDQUIST; HETHERINGTON, 1998; LIPINSKI et al., 2012). Além disso, algumas espécies podem utilizar ajustes temporários nos parâmetros acústicos frente ao ruído ambiente, como aumentar o número, a duração e a frequência dominante das notas (BRUMM; SLABBEKOORN, 2005; GRAFE et al., 2012). Adaptações e estratégias comportamentais na comunicação também têm sido atribuídas à pressão imposta por receptores ilegítimos que são fonotaticamente orientados até suas presas ou hospedeiros, como observado nas interações entre machos de anuros e morcegos predadores (TUTTLE; RYAN, 1981; HALFWERK et al., 2014), e entre machos de anuros e mosquitos parasitas do gênero *Corethrella* (BERNAL et al., 2006; BORKENT, 2008), respectivamente.

A comunicação visual evoluiu independentemente em diversas famílias de anuros e é complexa em espécies diurnas que habitam riachos (HÖDL; AMÉZQUITA, 2001; HARTMANN et al., 2005). Nesses casos, sinais visuais supostamente evoluíram para suplementar ou substituir a comunicação acústica, devido principalmente à pressão exercida pelo ruído ambiente. Entretanto, têm se constatado que muitas espécies também utilizam displays multimodais, caracterizados pela associação entre sinais acústicos e visuais e/ou sinais de outras modalidades sensoriais (GRAFE et al., 2012; PREININGER et al., 2013; STARNBERGER et al., 2014). A associação entre sinais pode ser simultânea ou sequencial e, dependendo do tipo de pressão seletiva que atua sobre os componentes, o display multimodal pode ter evoluído para cumprir diferentes funções nas interações sociais e interespecíficas (HEBETS; PAPAJ, 2005; HIGHAM; HEBETS, 2013).

Como exposto anteriormente, a comunicação acústica em anfíbios anuros está diretamente relacionada à reprodução e, consequentemente, ao sucesso reprodutivo individual (RYAN, 1980; GERHARDT, 1991; TÁRANO; HERRERA, 2003; TÁRANO; FUENMAYOR, 2013). Os padrões temporais de atividade de reprodução em anuros variam da atividade explosiva, quando eventos reprodutivos são desencadeados por um fator climático específico e duram poucos dias, à atividade prolongada e/ou contínua, quando a temporada reprodutiva se prolonga por vários meses ou ocorre ao longo do ano todo, respectivamente (CRUMP, 1974; WELLS, 1977). Exercem influencia sob os padrões de atividade reprodutiva das espécies os fatores intrínsecos, como ontogenia e ritmo circadiano (OISHI et al., 2004), e fatores extrínsecos, como as variáveis ambientais locais (WELLS, 2007). Muito do conhecimento sobre a atividade de vocalização de espécies de anuros neotropicais advém de estudos sobre comunidades de ambientes lênticos, em que a maioria das espécies possui atividade noturna. De maneira geral, estes estudos têm demonstrado que em áreas tropicais a atividade das espécies é fortemente influenciada pela variação na temperatura do ar e na precipitação (e.g., ZINA et al., 2007; SLUYS, VAN et al., 2012), enquanto que a atividade de comunidades subtropicais é influenciada principalmente pela variação na temperatura do ar e no fotoperíodo (e.g., BOTH et al., 2008; CANAVERO et al., 2008; CANAVERO; ARIM, 2009). Por outro lado, o conhecimento do efeito das variáveis ambientais na atividade de canto de anuros diurnos de riacho é ainda incipiente, e os poucos estudos existentes foram conduzidos em áreas tropicais e reportaram o efeito da temperatura do ar, da luminosidade e fotoperíodo sobre a atividade de canto (HATANO et al., 2002; ALMEIDA-GOMES et al., 2007).

Em estudos sobre os padrões de atividade reprodutiva de anuros, uma abordagem complementar à da atividade de vocalização dos machos advém da descrição dos ciclos gametogênicos de machos e fêmeas através de cortes histológicos das gônadas, da avaliação das relações entre tamanho do indivíduo e tamanho da gônada (relações tamanho x fecundidade), e do investimento reprodutivo de ambos os sexos ao longo de uma série temporal (e.g., PRADO et al., 2000; PRADO; HADDAD, 2005; KAEFER et al., 2007). Tal abordagem permite determinar com maior precisão o padrão temporal de reprodução de uma determinada espécie. Entretanto, a exemplo do que ocorre acerca dos padrões temporais de atividade de canto, pouco se conhece sobre os ciclos gametogênicos e estratégias de investimento reprodutivo de anuros diurnos de riachos da região neotropical.

1.3. *Crossodactylus schmidti* como modelo de estudo

A família Hylodidae Günther, 1858 é constituída por espécies predominantemente diurnas associadas a riachos de interior de mata, e está representada atualmente por 46 espécies agrupadas em três gêneros (FROST, 2015): *Hylodes* (25 espécies), *Crossodactylus* (14 espécies) e *Megaelosia* (sete espécies). Os três gêneros formam um clado reconhecido por vários estudos (NUIN; do VAL , 2005; FROST et al., 2006; GRANT et al., 2006; PYRON; WIENS, 2011). Grant et al. (2006) considerou Hylodinae como o táxon irmão de “Dendrobatidae + Aromobatidae”, retirando-o da família Cycloramphidae, onde havia sido alocado anteriormente por Frost et al. (2006). A mais recente análise filogenética de anfíbios, entretanto, sugere que Hylodidae é o táxon irmão de Alsodidae, não mais próximo de

“Dendrobatidae + Aromobatidae”, e que *Crossodactylus* é o táxon irmão de um clado composto por *Megaelosia* e *Hylodes* (PYRON; WIENS, 2011; FROST, 2015).

O gênero *Crossodactylus* é o segundo maior gênero da família Hylodidae, distribuindo-se do nordeste ao sul do Brasil, sul do Paraguai e norte da Argentina (FROST, 2015). Caramaschi e Sazima (1985) reconheceram três grupos de espécies no gênero *Crossodactylus*: o grupo *C. gaudichaudii*, com oito espécies reconhecidas, o grupo *C. trachystomus*, com duas espécies, e o grupo *C. schmidti*, composto apenas pela espécie nominal. Recentemente, Pimenta et al. (2014) revisaram o complexo *C. dispar*, constatando a existência de duas espécies válidas usadas sob este mesmo nome, e descreveram outras duas novas espécies que não puderam ser atribuídas aos grupos fenéticos propostos por Caramaschi e Sazima (1985). Assim, posicionaram-se contra o uso de grupos fenéticos para agrupar as espécies do gênero, dada a fragilidade dos estados e dos caracteres que anteriormente baseavam a formação dos grupos de espécies e a falta de uma análise filogenética exclusiva do gênero. A maioria dos estudos sobre espécies de *Crossodactylus* ainda é de cunho taxonômico e, até recentemente, o único trabalho de cunho comportamental foi o de Weygoldt e Silva (1992), sobre o comportamento de corte e de oviposição de *C. gaudichaudii*. Somente a partir de 2006 surgiram outros estudos abordando aspectos da história de vida de espécies de *Crossodactylus*, versando sobre atividade de canto (ALMEIDA-GOMES et al., 2007), variação acústica e morfológica (PIMENTA et al., 2008), uso do ambiente e dieta (JORDÃO-NOGUEIRA et al., 2006; ALMEIDA-GOMES et al., 2007; WACHLEVSKI et al., 2008), e dinâmica populacional de girinos (ALMEIDA-GOMES et al., 2012).

Crossodactylus schmidti (Figura 2) é a espécie que possui a distribuição mais subtropical dentre as espécies do gênero, sendo também a única a adentrar consideravelmente no interior do continente sul americano e a ocorrer exclusivamente em localidades de Floresta Estacional Semidecídua (*sensu* OLIVEIRA-FILHO et al. 2006) nos estados brasileiros do Paraná, Santa Catarina e Rio Grande do Sul (CALDART et al., 2010; LUCAS; GARCIA, 2011), chegando até o sul do Paraguai (BRUSQUETTI; LAVILLA, 2006) e ao nordeste da Argentina (GALLARDO, 1961). Desde 2011 *C. schmidti* consta oficialmente como espécie “CR - Criticamente Em Perigo” na lista da fauna ameaçada do estado de Santa Catarina (ESTADO DE SANTA CATARINA, 2011), e desde 2014 consta como “EN - Em Perigo” na lista da fauna ameaçada do estado do Rio Grande do Sul (ESTADO DO RIO GRANDE DO SUL, 2014). *Crossodactylus schmidti* é uma das espécies do gênero com mais informações disponíveis sobre distribuição geográfica e história natural. O primeiro registro da espécie para o Rio Grande do Sul foi realizado por Caldart et al. em 2010, e desde então diversos estudos foram realizados com as populações do Parque Estadual do Turvo, localizado no extremo noroeste do estado. Dentre as informações de história natural disponível está a descrição do sítio de vocalização (CALDART et al., 2010), dos cantos de anúncio e agressivo (CALDART et al., 2011a), dos predadores diurnos e noturnos (CALDART et al., 2011b) e a investigação da ecologia alimentar em relação à disponibilidade de presas (CALDART et al., 2012).

Figura 2. Macho de *Crossodactylus schmidti* vocalizando a partir do sítio de vocalização típico da espécie no Parque Estadual do Turvo, Rio Grande do Sul, Brasil.

Após alguns anos estudando o mesmo organismo e obtendo informações diversas sobre sua história natural, posso afirmar que *C. schmidti* é um bom candidato a figurar como um organismo modelo para estudos comportamentais sobre reprodução e comunicação em anfíbios anuros, especialmente dentre os anuros diurnos de riachos. Meu posicionamento se deve ao acúmulo de informações importantes sobre sua história natural e ao fato da espécie ter atividade predominantemente diurna, o que torna o registro das interações sociais e dos sinais facilitado em relação às espécies de atividade noturna. Hödl e Amézquita (2001) já haviam indicado os gêneros *Hylodes* e *Crossodactylus* como táxons de interesse em estudos sobre comunicação em anuros neotropicais, e o conhecimento acumulado sobre *C. schmidti* corrobora a sugestão desses autores. Nesse sentido, argumento ao longo dos capítulos desta tese que *C. schmidti* é um bom modelo para avaliar tanto questões relacionadas à estrutura e à função de sinais, dado seu amplo repertório de sinais e à relativa facilidade de registrá-los em hábitat natural, como questões relacionadas às pressões seletivas que influenciam na evolução de adaptações na estrutura e função dos sinais e no uso de estratégias comunicativas e reprodutivas. Konrad Lorenz (1981) escreveu sobre as diferenças entre os objetivos de pesquisa em Física e em Biologia, argumentando que para o físico, o entendimento das estruturas e funções dos sistemas é apenas um meio, um objetivo provisório no caminho rumo à abstração de leis mais gerais. Para o biólogo, contudo, o interesse nas estruturas e nas funções das coisas vivas, mesmo que levem a um entendimento parcial, é essencial:

> *"Investigadores de sistemas vivos empregam o mesmo método (...). Mas na sua busca do conhecimento, o objetivo não é o mesmo do físico: o biólogo deseja aprender a compreender o sistema vivo em si, por ele próprio, mesmo que seja apenas um sistema parcial. Todos os sistemas vivos interessam-no igualmente, independentemente dos seus níveis de integração, sua simplicidade ou sua complexidade. Assim como na análise do físico, o biólogo também procede do "topo" para o "fundo", do mais particular para o mais geral. Nós, biólogos, estamos também convencidos de que um único conjunto de leis mais gerais e mais especiais, em si mesmo destituído de contradição, governa o Universo. Destas leis, as mais especializadas podem, em princípio, ser reduzidas às mais gerais desde que se conheça a estrutura da realidade na qual vigora, assim, como a gênese histórica desta estrutura".*

Stephen Stearns (2000) também chamou a atenção para as diferenças entre biólogos evolutivos, biólogos moleculares e físicos, destacando que para o primeiro, as principais motivações das suas perguntas se devem à variação individual e as fontes que causam variação:

> *"Os biólogos evolutivos diferem dos biólogos moleculares e dos físicos em um importante objetivo básico. Os biólogos moleculares querem descobrir os mecanismos comuns a todos os seres vivos; eles se concentram no que é semelhante em geral a todas as células. Os físicos querem descobrir as propriedades gerais da matéria e energia, as leis válidas em todos os lugares e todos os tempos. Os biólogos evolucionistas fazem perguntas inspiradas por comparações de diferenças, diferenças entre espécies, entre as populações, entre os indivíduos. Eles querem entender por que as coisas são diferentes, e não por que não elas são as mesmas. Eles querem entender o que causa a diversidade. Grande parte do seu pensamento é colorida por esta concentração sobre as causas de variação".*

Dessa forma, utilizando *C. schmidti* para investigar questões sobre comunicação e reprodução – i.e., diversidade de sinais, estrutura dos sinais acústicos, relações sinal x ruído, associação entre sinais de diferentes modalidades, pressão de receptores ilegítimos na comunicação e efeitos das variáveis ambientais na atividade reprodutiva – podemos contribuir para o conhecimento sobre evolução de adaptações e de estratégias relacionadas à comunicação e reprodução num táxon particularmente interessante dentre os anuros: as rãs diurnas que habitam riachos.

1.4. Estrutura da tese

Esta tese está estruturada de acordo com as normas da 8ª edição da "Estrutura e apresentação de monografias, dissertações e teses: MDT", da Universidade Federal de Santa Maria (MDT, 2012). Compõe esta tese cinco capítulos redigidos no formato de artigos científicos, conforme descrição abaixo.

Capítulo I: **New records of *Crossodactylus schmidti* Gallardo, 1961 (Anura: Hylodidae) for the state of Rio Grande do Sul, Brazil, with data on morphometry and an updated geographic distribution map**

Neste capítulo apresento registros de novas populações de *C. schmidti* para o estado do Rio Grande do Sul, atualizo a distribuição conhecida para a espécie e discuto morfometria e potenciais ameaças à conservação das populações que ocorrem no Rio Grande do Sul.

Capítulo II: **Social interactions in a neotropical stream frog reveal a complex repertoire of visual signals and the use of multimodal communication**

Neste capítulo descrevo o repertório de sinais visuais e multimodais de *C. schmidti*, discutindo as potenciais causas e processos relacionados à evolução do repertório visual. Os sinais são descritos e caracterizados quanto ao contexto social em que são emitidos, ao sítio (microhábitat) utilizado para emissão, e aos padrões de associação entre sinais acústicos e visuais. Para isso, analisei filmagens das interações sociais de *C. schmidti* obtidas *in situ*.

Capítulo III: **Acoustic communication in a Neotropical diurnal stream frog (Anura, Hylodidae): temporal patterns of calling and noise-dependent adjustments of call traits**

Neste capítulo investigo os padrões diário e mensal de atividade de canto de *C. schmidti*, os fatores ambientais que influenciam a atividade de canto em ambas as escalas temporais, e as adaptações e estratégias acústicas utilizadas pelos machos para lidar com o ruído ambiente produzido pela água corrente dos riachos. Para isso, monitorei a abundância de machos em atividade de canto e o número de cantos emitidos diariamente e mensalmente, medi variáveis ambientais locais e descrevi a estrutura do sinal acústico e do ruído ambiente para avaliar as relações sinal x ruído e testar hipóteses relacionadas.

Capítulo IV: **Continuous reproductive cycle in males and females of *Crossodactylus schmidti* (Anura, Hylodidae) in the subtropical climate of southern Brazil**

Neste capítulo investigo a biologia reprodutiva de *C. schmidti* a partir da análise histológica de testículos e ovários de indivíduos coletados em todos os meses do ano. Para isso, descrevi o padrão temporal de ocorrência de indivíduos maduros, avaliando os ciclos gametogênicos de machos e fêmeas, as relações tamanho-fecundidade e investimento reprodutivo para ambos os sexos, e a ocorrência de dimorfismo sexual em características morfológicas externas.

Capítulo V: **Parasitic flies attracted to frog calls in the Austral Neotropics: evidence of the interaction and discussion on potential selective pressure**

Este capítulo deriva de parte dos resultados apresentados no Capítulo II, no qual descrevo a interação entre machos de *C. schmidti* em atividade de canto e mosquitos parasitas. Neste capítulo investigo a interação entre mosquitos parasitas de anuros e machos de anuros de espécies de habitats lênticos e lóticos, a fim de determinar quais táxons de parasitas são atraídos pelo sinal acústico até o macho hospedeiro. Para tal, realizei experimentos acústicos com armadilhas de sucção para coleta dos dípteros, a partir das quais foram emitidos cantos de três espécies de anuros típicas da área de estudo.

Os capítulos I e II encontram-se publicados nos periódicos *Check List* (9,6: 1552–1555, 2013) e *Behaviour* (151: 719–739, 2014), respectivamente. Para fins de padronização das normas de redação, os demais capítulos estão formatados segundo as normas do periódico *The American Naturalist*.

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CAPÍTULO I

New records of *Crossodactylus schmidti* Gallardo, 1961 (Anura: Hylodidae) for the state of Rio Grande do Sul, Brazil, with data on morphometry and an updated geographic distribution map

Check List 9(6): 1552–1555, 2013

New records of *Crossodactylus schmidti* Gallardo, 1961 (Anura: Hylodidae) for the state of Rio Grande do Sul, Brazil, with data on morphometry and an updated geographic distribution map

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ABSTRACT: *Crossodactylus schmidti* Gallardo, 1961 is a diurnal frog inhabiting rocky streams in forested areas, recently considered as a threatened species in the Brazilian states of Rio Grande do Sul and Santa Catarina. Herein we present four new population records of *C. schmidti* for the northwestern region of Rio Grande do Sul state and discuss the major impacts that may be threatening the species in the state. Additionally, we present an updated map of the current known species distribution and compare morphometric data among populations from Rio Grande do Sul state and Paraná state, in Brazil, and the province of Misiones, Argentina, based on the examination of voucher specimens available from herpetological collections.

Crossodactylus schmidti Gallardo, 1961 occurs in northeastern Argentina (Gallardo 1961; Cei 1980), southern Paraguay (Brusquetti and Lavilla 2006), and southern Brazil (Caldart *et al.* 2010; Lucas and Garcia 2011) at elevations of 300-750 meters (Frost 2012). It occurs in rocky streams in forested areas, where rocks are typically used as calling sites (Caldart *et al.* 2010). Studies on natural history and ecology of *C. schmidti* were nonexistent until recently (see Caldart *et al.* 2011; 2012; Bastiani *et al.* 2012), probably because of the lack of knowledge on its distribution. Recent extensions of its distribution indicate a fragmented distribution associated with the southern portion of the Atlantic Forest (Bastiani *et al.* 2012), a severely fragmented and threatened biome (MMA 2003; 2010; SOS Mata Atlântica 2011). Efforts to obtain new occurrence records of *C. schmidti* are important for the assessment of the threats and conservation status of this species, due to the prospect of an increasing reduction of riparian forests in Brazil as a result of changes in the Brazilian forest code, affecting associated reophilic species (Toledo *et al.* 2010). Moreover, *C. schmidti* was recently categorized as a threatened species in the Brazilian states of Santa Catarina (CONSEMA, 2011) and Rio Grande do Sul.

The first record of *C. schmidti* for the Brazilian state of Rio Grande do Sul was based on specimens collected in the Parque Estadual do Turvo, located in the municipality of Derrubadas (Caldart *et al.* 2010). After that, Machado (2012) expanded the species distribution for the municipality of Taquaruçu do Sul, in an area adjacent to the Reserva Indígena do Guarita, without voucher specimens. So far, these are the only two published records of populations of *C. schmidti* occurring in the state. In this communication we report the occurrence of *C. schmidti* for four additional municipalities in the northwestern region of Rio Grande do Sul, compare morphometric data among adult specimens from populations of

Misiones, Argentina, Rio Grande do Sul and Paraná, Brazil, through examination of voucher material from herpetological collections, and provide an updated map of the species distribution.

On September 12, 2010, two adult specimens were collected in the municipality of Braga, in a first order stream within an area of highly fragmented mesophytic semideciduous forest, surrounded by an agroecosystem matrix (27°36'4.66" S, 53° 47'20.89" W; 377 m altitude). The fragment has an area of about 3.900 m² and is located 1.3 km upstream of the Small Hydroelectric Power Plant (SHP) Marco Baldo (750 m in a straight line), 15.7 km upstream of SHP Toca do Tigre (4.5 km in a straight line), and 11.4 km downstream of SHP Carlos Gonzatto (4.5 km straight line), all located on the Turvo river. In the municipality of Dois Irmãos das Missões, on December 27 and 28, 2011, four specimens, two adults and two juveniles, were collected in a first order stream within the Reserva Biológica Municipal Moreno Fortes (27°36'39.58" S, 53°30'8.91" W, 493 m altitude), a fragment of 458.6 ha of mesophytic semideciduous forest. The remaining records occurred in fragments of mesophytic semideciduous forest in the municipalities of Iraí on March 7, 2012, where we collect a juvenile specimen in a first order stream tributary of the Mel river located within the Reserva Florestal Bosque Sagrado, a municipal fragment of about 1 ha (27°12'4.62" S, 53°14'52.94" W; 249 m altitude), and in the municipality of Frederico Westphalen on March 12, 2012, where a adult specimen was collected in the Tunas rivulet within the Parque Natural Municipal Arcângelo Busatto, an area of 6.6 ha of preserved forest located within a fragment of approximately 34 ha (27°22'29.39"S, 53°25'40.71" W; 453 m altitude).

These specimens were collected manually, killed with Xylocaine 5%, fixed in 10% formalin, preserved in 80% alcohol (licenses: ICMBio No. 31188-1; IBAMA No 035/2010, reg. 4945131), and deposited in the Herpetological Collection of the Universidade Federal de Santa Maria (ZUFSM 4860-4861, 5265, 5780-5784). The morphological measurements of the adult specimens collected, obtained with a digital caliper (accuracy of 0.01 mm), were compared with measurements of the holotype present in the description of the species (Gallardo 1961) and with those of adult specimens from Misiones, Argentina, Rio Grande do Sul, Brazil, and Paraná, Brazil, obtained from herpetological collections (CFBH, MZUSP, and ZUFSM). In general, morphological measures were similar among populations (Table 1). All specimens presented the diagnostic characteristics of *C. schmidti* according to the description of Gallardo (1961), such as the canthus rostralis less marked, the shorter snout, and a large interorbital distance. Although a high variation in snout-vent length (22.9-31.8 mm), tibia length (11.9-14.5 mm), and foot length (15.0-21.4 mm) between populations, it may be interpreted as a result of age or sexual differences between specimens, since Gallardo (1961) presented the snout-vent lenght in adults ranging from 21 to 31.5 mm.

Based on our literature survey and morphological analysis, the distribution of *C. schmidti* may be considered confirmed for 17 localities (Figure 1; Appendix 1). In Paraguay, the species' distribution includes only one locality so far, whereas for Argentina it includes the type locality and two additional locations. Chébez & Casanãs (2000) mentioned additional records related to the species' description (Gallardo, 1961) for the northern half of the Misiones province, but did not present geographic coordinates. In Brazil, the species' distribution is currently represented by six localities in the northwestern region of Rio Grande do Sul, three localities in western Santa Catarina, and four localities in Paraná state. For the state of Paraná, Caldart *et al.* (2010) mentioned the occurrence of *C. schmidti* for three localities, including the municipalities of Três Barras do Paraná, within the Parque Estadual Rio Guarani (Segalla *et al.* 2004; P.C.A. Garcia, pers. com.), Maringá, and Porto Camargo. After that, the records mentioned by Caldart *et al.* (2010) for the municipalities of Porto Camargo and Maringá were considered not confirmed by Bastiani *et al.* (2012), since they were based on museum records without examination of the material. The record for Porto Camargo mentioned by Caldart *et al.* (2010) derived from specimens collected prior to the description of the species, deposited in the Museu de Zoologia, Universidade de São Paulo (MZUSP 15855-15863) and identified as *C. schmidti* by D. Cochran. Subsequently, part of this material (MZUSP 15855-15856) was exchanged with foreign museums (C. Mello, pers. com.). Recently, we examined the remaining specimens collected in Porto Camargo (MZUSP 15857-15858, 15860-15863) and the specimens of Maringá (CFBH 17174-17178, 17265) from which we could confirm their identities as *C. schmidti*. In addition, another record for Paraná state was found based on a museum specimen (CFBH 19842), collected in the municipality of Bandeirantes, within the Parque Estadual Mata São Francisco (Table 1). Regarding the historical record of *C. schmidti* in Porto Camargo, collection efforts at this location are important to verify if this population still persists.

Overall, the geographical distribution of *C. schmidti* seems to be associated with the Misiones Nucleus of the Seasonally Dry Tropical Forests and its enclaves and transitional areas (*sensu* Werneck *et al.* 2011). Indeed, a recent study has considered *C. schmidti*, along with *Hypsiboas curupi* and *Proceratophrys avelinoi*, as a frog species endemic to the Misiones Nucleus and its transitional areas of Seasonal Forests (Iop *et al.* 2011). Furthermore, for conservation purposes it is important to highlight that the geographical distribution of *C. schmidti* herein proposed is also entirely included within the Alto Paraná Atlantic Forest

Ecoregion and its transitional areas with the Araucaria Moist Forest Ecoregion (*sensu* Olson *et al.*, 2001). *Crossodactylus schmidti* is considered as "near threatened" in the Red List of Threatened Species Worldwide (IUCN 2012) because of threats such as the reduction of forests and pollution of soil and water due to agricultural practices (Segalla *et al.* 2004). Results about habitat use suggest that *C. schmidti* is a habitat specialist (Caldart *et al.* 2010; Bastiani *et al.* 2012) and probably sensitive to environmental disturbances (Segalla *et al.* 2004). Bastiani *et al.* (2012) warned of the risk of extirpation of *C. schmidti* in Santa Catarina state, as a result of the progressive reduction of riparian forests in the western region of the state and the absence of populations at sites where it would be likely to occur.

For the state of Rio Grande do Sul, our additional records suggest that the distribution of *C. schmidti* must be greater than currently known. Nevertheless, the northwestern region of the state suffers similar impacts to those that may be threatening the species in Santa Catarina, i.e., the reduction of forests, expansion of agricultural crops, and hydroelectric power plants. The deforestation of riparian forests may cause critical increase in the water temperature of streams and, therefore, may have a consequent effect on the persistence of species sensitive to high thermal variations. Among 11 species from the subtropical Atlantic Forest of Misiones, Argentina, larvae of reophilic species such as *C. schmidti* and *Hypsiboas curupi* were the most sensitive to thermal variation in water temperature, presenting lower values of upper thermal tolerance (Duarte *et al.* 2011).

Except for the occurrence of *C. schmidti* in the Parque Estadual do Turvo (Caldart *et al.* 2010), in the surroundings of the Reserva Indígena do Guarita (Machado 2012), and in the Reserva Biológica Municipal Moreno Fortes, which have considerable remnants of semideciduous forest, the new records come from fragments located within municipal recreational parks (i.e., Iraí, Frederico Westphalen) or from small fragments in poor conservation condition and strongly affected by human-induced impacts (i.e., Braga). Although our new population records did not expand considerably the geographic distribution of *C. schmidti* in Rio Grande do Sul, we believe they are very opportune because they highlight the importance of conserving these habitats and the need of further studies to verify whether the populations of *C. schmidti* may be declining in these areas, as well as to localize additional populations.

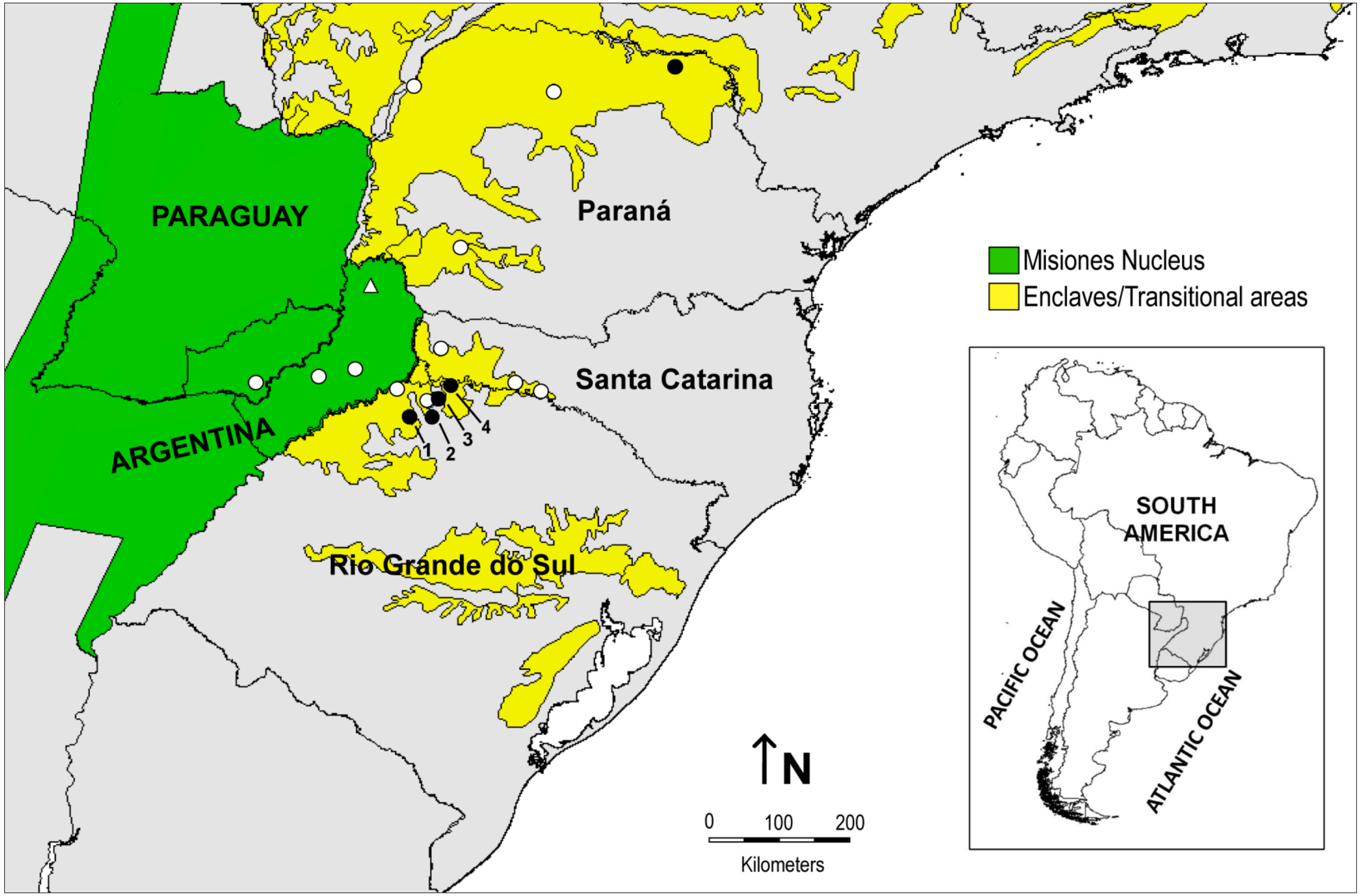

Figure 1. Geographical distribution of *Crossodactylus schmidti* associated to Seasonally Dry Tropical Forests of the Misiones Nucleus and Enclaves/Transitional areas (*sensu* Werneck *et al.* 2011). Triangle: type locality, Yacú-poí, Misiones, Argentina. White circles: previously known records of *C. schmidti* (see details of each record in the Appendix 1). Black circles in Rio Grande do Sul, Brazil: new records of *C. schmidti* for the northwestern region of the state: 1 – municipality of Braga (27°36'4.66" S, 53° 47'20.89" W); 2 – municipality of Dois Irmãos das Missões (27°36'39.58" S, 53°30'8.91" W); 3 – municipality of Frederico Westphalen (27°22'29.39"S, 53°25'40.71" W); 4 – municipality of Iraí (27°12'4.62" S, 53°14'52.94" W). Black circle in Paraná, Brazil: additional record for the state based on a voucher specimen (CFBH 19842) collected in the municipality of Bandeirantes (23°6'40.28"S, 50°22'16.90"W).

Table 1. Morphometric comparison (mm) of adults of *Crossodactylus schmidti* from populations of the province of Misiones, Argentina, and the states of Rio Grande do Sul and Paraná, Brazil [1]. Values are presented as mean, followed by standard deviation and range in parenthesis, when available. SVL (snout-vent length); HW (head width); HL (head length); ED (eye diameter); ED/HL (eye diameter-head length ratio); IOD (interorbital distance); IND (internostril distance); END (eye-nostril distance); TD (tympanum diameter); THL (thigh length); TBL (tibia length); FL (foot length).

	Misiones, Argentina		Rio Grande do Sul, Brazil				Paraná, Brazil		
	Holotype (n=1)	San Vicente (n=2)	Derrubadas (n=11)	Dois Irmãos das Missões (n=2)	Braga (n=2)	Frederico Westphalen (n=1)	Porto Camargo (n=4)	Maringá (n=2)	Bandeirantes (n=1)
SVL	29.0	(24.1-24.9)	28.1 ± 2.6 (24.4-31.8)	(27.9-29.2)	(25.1-26.4)	24.7	25.7 ± 1.1 (24.8-27.3)	(22.9-26.5)	28.8
HW	10.0	(8.2-8.8)	8.9 ± 0.7 (8.2-10.2)	(8.5-9.5)	(8.0-9.0)	7.9	9.1 ± 0.9 (8.3-10.4)	(8.6-9.1)	9.4
HL	9.0	(8.7-9.2)	9.1 ± 0.7 (8.0-10.4)	(9.4-9.5)	(8.3-9.0)	8.8	9.4 ± 0.4 (9.0-9.9)	(9.0-9.5)	10.1
ED	4.0	(3.3-3.4)	3.2 ± 0.2 (2.9-3.6)	(3.1-3.1)	(3.2-3.6)	2.4	3.2 ± 0.1 (3.1-3.3)	(3.4-3.5)	4.0
ED/HL	0.44	(0.37-0.38)	0.35 ± 0.02 (0.31-0.39)	(0.33-0.33)	(0.39-0.40)	0.27	0.34 ± 0.01 (0.33-0.35)	(0.33-0.37)	0.39
IOD	4.0	(4.9-5.4)	5.0 ± 0.5 (4.3-5.8)	(4.2-4.3)	(4.3-4.4)	4.7	5.2 ± 0.1 (5.1-5.3)	(5.4-5.6)	6.0
IND	3.5	(2.9-3.1)	2.9 ± 0.3 (2.5-3.4)	(2.9-3.5)	(2.7-3.0)	2.8	3.0 ± 0.1 (2.9-3.2)	(2.8-3.2)	3.5
END	2.5	(1.9-2.0)	2.5 ± 0.3 (2.1-3.0)	(2.3-2.3)	(2.4-2.5)	2.8	2.0 ± 0.2 (1.8-2.2)	(2.1-2.2)	2.1
TD	2.0	(1.6-1.8)	2.3 ± 0.2 (2.0-2.7)	(2.2-2.2)	(2.3-2.4)	2.1	1.8 ± 0.2 (1.5-1.9)	(1.8-1.9)	2.2
THL	12.5	(11.8-12.0)	12.2 ± 0.6 (11.4-13.2)	(11.9-12.5)	(11.7-12.4)	11.1	11.7 ± 0.4 (11.2-12.1)	(12.3-13.5)	13.9
TBL	14.0	(11.9-12.2)	13.0 ± 0.8 (11.9-14.2)	(12.2-12.3)	(12.1-13.2)	12.6	12.9 ± 0.6 (12.1-13.3)	(12.1-13.3)	14.5
FL	15.0	(17.6-18.3)	19.2 ± 1.2 (17.6-21.4)	(17.3-18.1)	(18.3-18.8)	18.5	18.9 ± 0.6 (18.1-19.4)	(18.3-20.0)	21.4

[1] Adult specimens examined, with their respective localities and collection numbers: Holotype (Gallardo, 1961), Yacú-poí, 30 km E of Puerto Libertad: MACN 2943; San Vicente: CFBH 9496-9497; Derrubadas: ZUFSM 4670, 4672, 4675-4678, 4680-4681, 4683, 4689, 4691; Porto Camargo: MZUSP 15857-15858, 15860, 15862; Maringá: CFBH 17176, 17265; Bandeirantes: CFBH 19842.

Acknowledgements

We are grateful to Célio F.B. Haddad and Taran Grant for allowing examination of specimens under their care, from the CFBH and MZUSP collections, respectively, and to Fernanda P. Werneck for kindly providing the STDF layers used to generate the distribution map. VMC and SI thank CAPES for the doctoral fellowships, and SZC is grateful to CNPq for the award of a research fellowship (no. 304929/2012-3).

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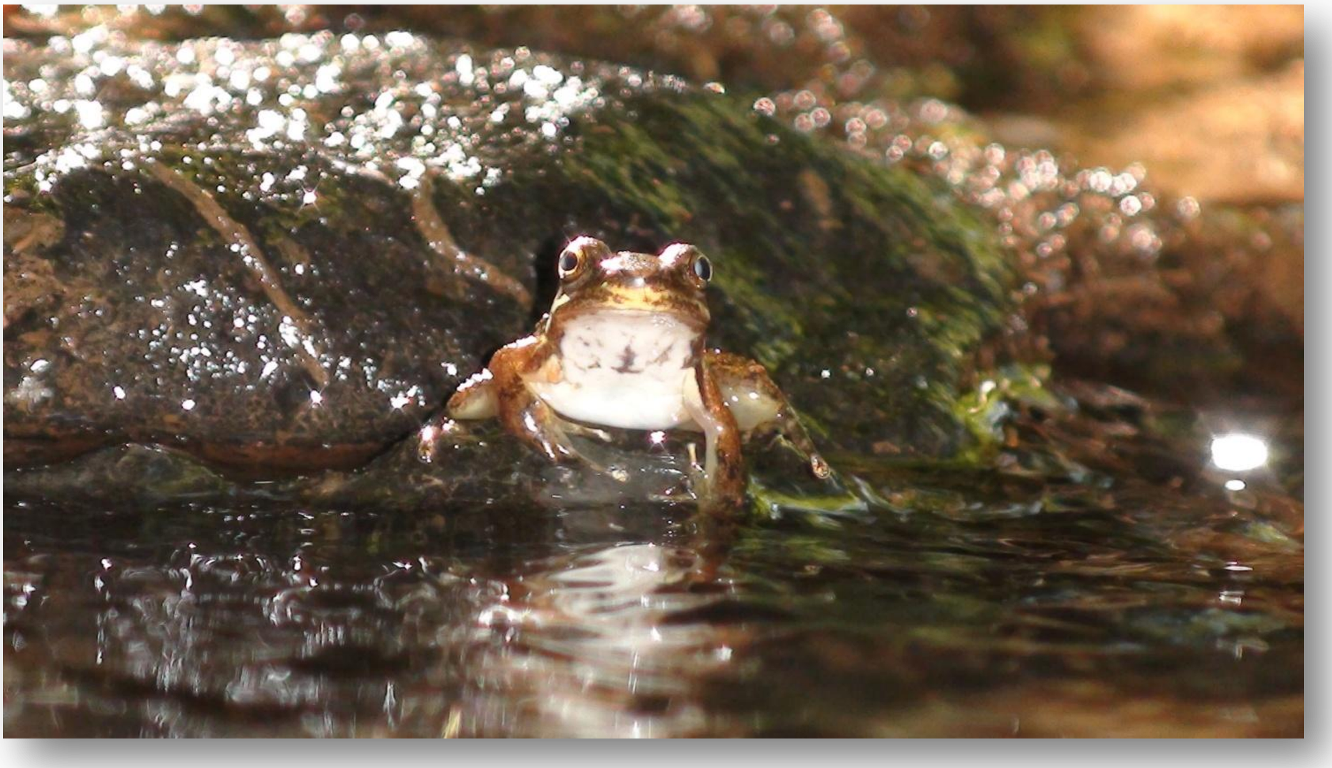
APPENDIX 1. List of published and collections records of *Crossodactylus schmidti* for Argentina, Paraguay, and for the Brazilian states of Rio Grande do Sul, Santa Catarina, and Paraná, which were used to generate the distribution map, with their respective coordinates and references. The records are listed below from western to eastern locations within their respective countries and states, to facilitate interpretation of the distribution map.

PARAGUAY: Itapúa, near El Tirol, 19,6 km (by road on Route 6) NNE the municipality of Encarnación, 27°10'26.85" S, 55°46'42.10" W (Brusquetti and Lavilla 2006; proximate coordinates, taken from free software Google Earth™). **ARGENTINA:** municipality of Aristóbulo del Valle, Cunã Pirú, Misiones, 27°05'14.52" S, 54°57'1.41" W (Duarte *et al.* 2011); municipality of San Vicente, Depto. Guarany, Misiones, 26°59'42.23" S, 54°29'8.41" W (Caldart *et al.* 2010; based on the voucher specimens CFBH 9495-9498; coordinates from the city proper, taken from free software Google Earth™); Yacú-poí, 30 km E of municipality of Puerto Libertad, Misiones, type locality, 25°55'29.09" S, 54°16'24.90" W (Gallardo 1961; proximate coordinates, taken from free software Google Earth™). **PARANÁ:** municipality of Porto Camargo, 23°22'2.32" S, 53°44'53.90" W (Caldart *et al.* 2010; based on the voucher specimens MZUSP 15857-15858, 15860-15863; coordinates from the city proper, taken from free software Google Earth™); municipality of Três Barras do Paraná, within the Parque Estadual Rio Guarani, 25°25'60" S, 53°7'60" W (Segalla *et al.* 2004; proximate coordinates, taken from free software Google Earth™); municipality of Maringá, at Fazenda Cesumar, 23°25'31.08" S, 51°56'18.96" W (Caldart *et al.* 2010; based on the voucher specimens CFBH 17174-17178, 17265; coordinates from the city proper, taken from free software Google Earth™); municipality of Bandeirantes, within the Parque Estadual Mata São Francisco, 23°6'40.28" S, 50°22'16.90" W (present study, based on the voucher specimen CFBH 19842;

coordinates from the city proper, taken from free software Google Earth™). **SANTA CATARINA:** municipality of São Miguel do Oeste, 26°44'41.3" S, 53°23'40.9" W (Lucas and Garcia 2011); municipality of Seara, 27°09'49" S, 52°25'27" W (Lucas and Garcia 2011); municipality of Concórdia, at the Parque Estadual Fritz Plaumann, 27°17'36" S, 52°06'38" W (Bastiani *et al.* 2012). **RIO GRANDE DO SUL:** municipality of Derrubadas, within the Parque Estadual do Turvo, 27°14'34.08" S, 53°57'13.75" W (Caldart *et al.* 2010, based on the voucher specimens ZUFSM 4670, 4672, 4675-4678, 4680-4681, 4683, 4689, 4691); municipality of Braga, 27°36'4.66" S, 53°47'20.89" W (present study, based on the voucher specimens ZUFSM 4860-4861); municipality of Taquaruçu do Sul, near the Reserva Indígena do Guarita, 27°24'53" S, 53°32'59" W (Machado 2012); municipality of Dois Irmãos das Missões, 27°36'39.58" S, 53°30'8.91" W (present study, based on the voucher specimens ZUFSM 5781-5784); municipality of Frederico Westphalen, 27°22'29.39" S, 53°25'40.71" W (present study, based on the voucher specimen ZUFSM 5265); municipality of Iraí, 27°12'4.62" S, 53°14'52.94" W (present study, based on the voucher specimen ZUFSM 5780).

CAPÍTULO II



Social interactions in a neotropical stream frog reveal a complex repertoire of visual signals and the use of multimodal communication

Behaviour 151 (2014) 719–739

Social interactions in a neotropical stream frog reveal a complex repertoire of visual signals and the use of multimodal communication

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Accepted 18 November 2013; published online 17 January 2014

Abstract

Visual communication has evolved independently in several families of frogs and is complex in diurnal stream frogs. In addition to isolated visual and acoustic signals, some species may emit multimodal displays characterized by the association of these signals. Through the analysis of focal recordings obtained in situ in southern Brazil, we investigated the visual and multimodal communication in the diurnal stream frog *Crossodactylus schmidti*. We found a complex visual repertoire of nine signals used in intraspecific communication, of which the signal Both legs kicking was described for the first time. Males, females and juveniles emitted visual signals, but males had a larger repertoire and used most signals in agonistic interactions. The four most common visual signals, Toe flagging, Limb lifting, Body jerking and Running-jumping, were emitted predominantly upon rocks in the stream bed. The emission of isolated visual and acoustic signals, as well as audiovisual displays with simultaneous or temporally coupled components, demonstrates that the communication in *C. schmidti* is complex and that the multimodal displays may have different functions. We discuss the historical, behavioural, ecological factors which may have influenced the evolution of the visual repertoire of *C. schmidti*, and how the ritualization of derived activities similar to visual signals may have led to the evolution of part of the repertoire. This study provides novel results on visual and multimodal communication for the genus *Crossodactylus* and demonstrates that *C. schmidti* represents an appropriate model for studies of complex displays.

Keywords

visual signal, acoustic signal, multimodal display, signalling environment, ritualization, derived activities, Hylodidae, *Crossodactylus*.

1. Introduction

Signals are the basic building blocks of communication (Hebets & Papaj, 2005) and can be defined as an act or structure that affects the behaviour of another organism, having evolved because of those effects, and which are effective because the effect has evolved to be affected by such act or structure (Scott-Phillips, 2008). Through the information conveyed by signals, an individual can evaluate attributes of others (Endler, 1993) during various inter- and intra-specific interactions (Enquist et al., 2010), and the completion of corresponding signals and responses is defined as the communication process (Scott-Phillips, 2008).

Acoustic communication plays a fundamental role in the life history of amphibians (Wells, 1977; Gerhardt, 1994) and is also important in a wide variety of animal taxa (Rosenthal & Ryan, 2000; Gerhardt & Huber, 2002). The communication through visual signals is another way to transmit information in a variety of taxa (Rosenthal & Ryan, 2000), which include the bioluminescent signal in fireflies (Takatsu, 2012), chela movements in crabs (Muramatsu, 2011) and the limb signals performed by frogs (Lindquist & Hetherington, 1998; Haddad & Giaretta, 1999). Studies on visual communication in frogs have found visual signals in species from different families, indicating that visual communication has evolved independently (Hödl & Amézquita, 2001). Vivid visual displays, such as the sexual dichromatism in males of the bufonid *Incilius luetkenii* during reproductive aggregations, illustrate how visual communication in frogs may be more important than previously recognized (Doucet & Mennill, 2009).

In the neotropical region several species of frogs emit visual signals, notably diurnal species that inhabit streams (Lindquist & Hetherington, 1998; Haddad & Giaretta, 1999). Diurnal activity and presence in acoustically complex signalling environments are considered factors that favoured the evolution of visual communication in frogs (Hödl & Amézquita, 2001), since for a signal to be detected there must be an increase in the signal-to-noise ratio, usually acquired by contrasting the signal with the characteristics of the surrounding environment (Endler, 1992). For diurnal species inhabiting streams, the contrast could be achieved, for example, by signalling from exposed sites such as rocks available in the streams (e.g., Narvaes & Rodrigues, 2005; Grafe & Wanger, 2007). Besides signalling from rocks during courtship and advertisement displays, rocks are also the stages for intense close-range agonistic interactions between males, in which acoustic and visual signals are employed Weygoldt & Carvalho-e-Silva, 1992; Haddad & Giaretta, 1999). Recent studies have drawn attention to the role of agonistic interactions in the evolution of visual signals, suggesting some signals may have evolved through the ritualization of aggressive movements performed during these combats (Giasson & Haddad, 2006; Preininger et al., 2013).

More recently, studies of frog communication have also investigated the association between visual and acoustic signals, i.e., multimodal communication. Multimodal displays are present in many taxa, e.g., crustaceans (Hebets & Rundus, 2011) and spiders (Elias et al., 2010; Wilgers & Hebets, 2012), and in frogs usually involves conspicuous visual and acoustic components emitted simultaneously (Rosenthal et al., 2004) or temporally coupled (Grafe & Wanger, 2007). The Neotropical genera of frogs *Hylodes* and *Crossodactylus* (Hylodidae) are considered taxa of interest for studies on the evolution of visual communication (Hödl & Amézquita, 2001), as some species of both genera could serve as subjects for observational and experimental studies on visual, acoustic, and multimodal communication because of their complex visual and acoustic repertoires (Weygoldt & Carvalho-e-Silva, 1992; Haddad & Giaretta, 1999; Hartmann et al., 2005b) whose signals can be recorded in situ. However, despite their undeniable importance, studies on communication of species of *Hylodes* and *Crossodactylus* have investigated the acoustic and visual signals separately, so that knowledge about multimodal communication in these taxa is still incipient. Furthermore, both visual and acoustic communication has been best studied in *Hylodes* species (Haddad & Giaretta, 1999; Wogel et al., 2004; Narvaes & Rodrigues, 2005).

Fundamental to understanding the evolution of visual signals are detailed descriptions of the behaviours as well as the social contexts in which the signals are employed (Amézquita & Hödl, 2004). In this study we describe the visual and multimodal communication in the neotropical stream frog *Crossodactylus schmidti*, and discuss the potential causes and processes related to the evolution of its visual repertoire. We performed a detailed investigation of the visual and multimodal signals of the species through the analysis of focal recordings obtained in situ, in order to address the following aims: (1) to characterize the repertoire of visual signals; (2) to identify the social contexts of visual signals emission; (3) to investigate the patterns of site use for visual signal emission, testing the hypothesis that visual signals are predominantly emitted from exposed sites within the signalling environment; (4) to characterize multimodal displays, through the investigation of the patterns of association between visual and acoustic signals; and (5) to describe the occurrence of derived behaviours similar to visual signals, emitted in nonsocial contexts.

2. Material and methods

2.1. Study site and species

The study was conducted at the Parque Estadual do Turvo (PET, 27°14’ 34.08” S, 53°57’ 13.74” W, 376 m a.s.l.), located in the municipality of Derrubadas, northwest of the state of Rio Grande do Sul, Brazil. The park covers an area of 17 491.4 ha of mesophytic semideciduous forest (sensu Oliveira-Filho et al., 2006) and is considered one of the last large remnants of this vegetation type in southern Brazil (SEMA, 2005). The local climate is characterized as subtropical sub-humid with a dry summer, an annual rainfall of 1787 mm, a mean annual temperature of 18.8°C and a mean temperature of the coldest month of 13.3°C (ST SB v climate type of Maluf, 2000).

Crossodactylus schmidti belongs to the family Hylodidae and occurs at elevations of 300–750 m in Misiones, Argentina, in southeastern Paraguay, and in western Paraná, western Santa Catarina and northern Rio Grande do Sul, Brazil (Frost, 2013).Crossodactylus schmidtiis predominantly diurnal and inhabits first or second order rocky streams, in which males use rocks as calling sites (Caldart et al., 2010). With regards to the species communication, two types of vocalizations are known, the advertisement and the aggressive calls (Caldart et al., 2011).

2.2. Behavioural observations

We investigated the visual communication of *C. schmidti* in three streams of PET between November 2009 and March 2010, and September 2011 and March 2012, months propitious for in situ observation of the species’ behaviour in the study area. Ad libitum observations (Lehner, 1996) were employed in the initial phase of the study for familiarization with behaviours and primary qualitative definition of the visual signals, totalling 20 h. Subsequently, focal observations (Lehner, 1996) were used for 1–3 days per month at times between 05.00 h and 19.00 h, the interval that comprises the period of greatest calling activity for the species (Caldart, unpublished data). In focal observations, continuous recordings over 1–30 min were made depending on the activity of the focal individual, i.e., the end of the session was determined by the departure of the focal individual from the observer’s visual field.

In total, 22 h of focal observations were recorded with a digital camcorder, always by the same observer (first author) from the margin of the streams. The recorded material consisted on focal observations of males (*N* = 59), females (*N* = 18) and juveniles (*N* = 12). Based on these recordings, the following parameters were subsequently determined for each focal observation: (1) the sex of the individual, (2) the relative frequency of each visual signal and the average duration of the most common visual signals, (3) the social context of the visual signal emission, (4) the site used for the emission of the most common visual signals, (5) the occurrence of multimodal displays emitted by males and (6) the occurrence of derived behaviours similar to visual signals, emitted in non-social contexts. Considering the ad libitum observations of the initial phase, the focal observations recorded with camcorder and the occasional ad libitum observations pre-or post-focal observations, the effort expended in observations of the behaviours totalled 52 h.

As *C. schmidti* do not present obvious sexual dimorphism, males were identified by calling activity, females identified only when ovigerous (obviously larger than males), and juveniles classified as all individuals clearly smaller than adults (i.e., snout-vent length ≤ 20 mm). The average duration of the most common visual signals were measured by frame-by-frame analysis of the behaviour (accuracy 0.033 s), starting as soon as motion was detected and ending when the initial state/posture was recovered. To avoid a possible classification of epiphenomena and derived activities as visual signals (see last paragraph in this section), we consider as visual signals only redundant, conspicuous and stereotyped movements or postures observed during social interactions (sensu Hödl & Amézquita, 2001; Hartmann et al., 2005a).

With regards to the social context of the visual signals, the interactions were classified as: (1) agonistic, aggressive interactions between conspecific males or between adults and juveniles at short or medium distances; and (2) courtship, interactions between males and females at short or medium distances. Short-distance interactions usually occurred between individuals up to approx. 50 cm apart and could involve tactile stimuli, whereas medium distance interactions usually occurred between individuals > 50 cm apart and did not involve tactile interactions. Long-distance interactions between individuals (i.e., distance > 2 m) were not considered when determining the social context of the signals, as the visual scope of the observer and the image quality of the camera could cause bias in the determination.

The sites used for emission of the most common visual signals were classified according to five types of substrates used in the stream bed/banks: (1) on rock, above the water level: the individual signals upon rocks in the stream bed, with all limbs visible above the water layer; (2) on rock, at the water level: the individual signals upon rocks in the stream bed, with all limbs visible at the water layer; (3) on rock, below the water level: the individual signals upon rocks in the stream bed, with all limbs below the water layer; (4) on vegetation, at water level: the individual signals upon vegetation in the stream bed, with all members visible at the water layer; (5) on marginal vegetation: the individual signals upon vegetation on the stream bank (e.g., Cyperaceae, Pteridophyta).

To evaluate the multimodal communication of males, displays were characterized as involving simultaneous visual and acoustic signals, or the temporally close emission of signals from both modalities. Considering that acoustic signals quickly degrade in noisy environments, ten seconds pre-or post-acoustic signal were used for characterizing multimodal displays with sequential components. The analysis of multimodal displays, specifically, was based on representative focal recordings of the acoustic activity and visual displays of advertising males (N = 2), agonistic interactions between males (N = 22) and males in courtship interactions (N = 12), totalling 6 h selected from the total recording time. Finally, the non-social derived activities similar to visual signals were classified into four categories: (1) foraging, motions performed or postures adopted during foraging activities; (2) ingestion of prey, motions performed or postures adopted during the ingestion of prey; (3) locomotion, motions performed or postures adopted during locomotion; (4) removal of flies, motions performed or postures adopted during interactions with parasitic flies. Although very similar to visual signals, such behaviours were considered as derived activities similar to the signals because of the obvious non-social context of the behaviour.

2.3. Statistical analysis

The G-test (Gotelli & Ellison, 2011) was used to test for possible differences in the emission of visual signals in relation to the generic type of site (rocks or vegetation). The same test was used to evaluate differences in the vertical use of rocks (with respect to the water level) as sites for emission of visual signals. The Chi-square test was used to compare the frequencies of visual signals, either isolated or associated with acoustic signals, and to compare the frequencies of visual signals associated with advertisement or aggressive calls.

All tests were performed in BioEstat 5.0 (Ayres et al., 2007) with a significance level of $p < 0.05$, from the total signalizations recorded for the most common visual signals (i.e., Toe flagging, Limb lifting, Body jerking and Running-jumping). The mosaic graph of signals distribution within emission sites of the signalling environment was made in the R software v. 2.12.2 (R Development Core Team, 2011).

3. Results

3.1. *Repertoire of visual signals*

Crossodactylus schmidti had a large repertoire of visual signals (Table 1). Males emitted nine visual signals, five were emitted by females, and three by juveniles. On average, males emitted more visual signals per focal observation (13 ± 34; 1–190) than females (3 ± 2; 1–8) or juveniles (5 ± 5; 1–12). Most of the visual signal types (eight) were observed during variable agonistic interactions between males, which may explain the high amplitude on the number of signal emission observed for males. However, females, males and juveniles also used visual signals during agonistic interactions between adults and juveniles. Signals observed exclusively during agonistic interactions were Leg kicking, Toe trembling, and a previously unreported signal emitted by males and juveniles, which we nominate Both legs kicking (see Supplementary video 1 in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/ journals/1568539x), described as follows: raising in a very fast movement both hind legs above the substrate, stretching and retracting them quickly; during the movement only the forelimbs maintain contact with the substrate. Six signals were emitted during courtship interactions but none were exclusively observed in this context.

Toe flagging was the most common signal (65%, *N* = 460), followed by Limb lifting (21%, *N* = 148), Body jerking (6%, *N* = 46) and Running-jumping (3%, *N* = 20). Limb lifting and Toe flagging were displayed very quickly; the mean duration of Toe flags was 0.386 ± 0.078 s (0.201–0.567 s, *N* = 61) and Limb lifting was 0.301 ± 0.079 s (0.134–0.567 s, *N* = 38). Signals emitted through bodily postures or movements, such as Body jerking and Running-jumping, were more variable in duration; Body jerking had a mean duration of 8.1 ± 3.7 s (3.6–13 s, *N* = 8), and Running-jumping 8.1 ± 3.5 s (3.7–15 s, *N* = 9). Limb liftings with the forelimbs were more frequent with the right limb (64 vs. 31 signalizations), while Limb liftings with the hindlimbs were more frequent with the left limb (40 vs. 13).

Considering the four most frequent signals, there were significant differences ($G = 13.1$, df = 3, $p = 0.005$) regarding the use of rocks and vegetation as sites for emission of visual signals (Figure 1). These were typically displayed upon rocks (91.7%, *N* = 617) and, less frequently, upon vegetation (8.3%, *N* = 56) (Figure 1). There were also significant differences regarding the vertical use of rocks (in relation to the water level) as sites for emission of visual signals ($G = 262.6$, df = 6, $p < 0.0001$). The emission of visual signals from rocks above the water level was more frequent (71.2%, *N* = 479) than from rocks at the water level

(11.7%, *N* = 79) or from rocks below the water level (8.8%, *N* = 59). Nevertheless, while emissions of Toe flagging and Body jerking were more common from rocks above the water level, emissions of Limb lifting and Running-jumping were more evenly distributed within the vertical categories of rocks in the stream bed.

3.2. Association between visual and acoustic signals

Toe flagging, Body jerking and Running-jumping emitted by males were more often associated with acoustic signals than isolated ($\chi^2 = 33.04$; df = 1; $p < 0.0001$; Figure 2). Multimodal displays involving these signals and calls were emitted exclusively simultaneously. Less often, Body lowering, Both legs kicking, Toe trembling and Upright posture were also emitted simultaneously with vocalizations. Unlike, Limb liftings were emitted more frequently isolated of acoustic signals than associated with them ($\chi^2 = 172.9$; df = 1; $p < 0.0001$; Figure 2), and when observed associated with calls (*N* = 10) were emitted before (3 s, *N* = 1), during (*N* = 4) or after the acoustic signal (5.8 ± 1.5 s, *N* = 5).

When emitted in a multimodal display, the visual signals were more often associated with aggressive calls than with advertisement calls ($\chi^2 = 171.3$; df = 1; $p < 0.0001$). The most frequent multimodal display consisted on the simultaneous emission of Toe flagging and aggressive calls, commonly observed during agonistic interactions between males. However, despite the clear association between some visual components and calls during intraspecific interactions, visual and acoustic signals were also widely used in a unimodal way in different social interactions (Table 2).

3.3. Derived behaviours similar to visual signals

Movements and postures similar to the signals Upright posture and Limb lifting were observed during clearly non-social contexts. A bodily posture similar to the signal Upright posture (*N* = 12) was observed in individuals engaged in foraging activity. In these situations, individuals adopted a posture similar to the visual signal when searching for prey located above them, stretching their limbs, keeping the head up and making the ventral and gular white colouring visible. The posture could be maintained for some time until the individual jumped toward the prey. Most of the derived behaviours, however, involved movements similar to the signal Limb lifting performed during contexts of prey ingestion, locomotion and removal of parasites. During the ingestion of large prey, we observed individuals performing rapid up-anddown movements with one of the forelimbs close to the mouth (*N* = 11), presumed to facilitate the intake of the prey. Fast lifting movements of the forelimbs were also observed during locomotion (*N* = 6), e.g., a sudden change in direction of the body to the right started with a quick raising of the right forelimb.

The most notable example of a derived behaviour similar to the Limb lifting signal was performed by a male in calling activity upon a rock at the water level, while being attacked by parasitic flies. The interaction occurred on September 22, 2009 at 13.05 h, and over the period of 8 min and 35 s, immediately after the male emitted calls, two flies landed on the male at least 26 times, mainly around the head and eyes, causing immediate responses in the form of fast up-and-down forelimb movements (see Supplementary video 2 in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x). During the interaction the male performed 22 quick movements very similar to the Limb lifting used in intraspecific communication. Seven movements were performed with the right forelimb immediately after flies landed on the right eye, nine were performed with the left forelimb immediately after they landed on the left eye and another six movements were also performed in the presence of the flies.

Table 1. Repertoire of visual signals of *Crossodactylus schmidti* observed at the Parque Estadual do Turvo, northwest of Rio Grande do Sul, Brazil, highlighting the sex and the social context of signal emission.

Visual signals		Sex	Courtship context	Agonistic context		
				M vs. M	M vs. J	F vs. J
Limbs	Leg kicking	M	-	M	-	-
	Both legs kicking	M, J*	-	M	-	-
	Limb lifting	M, F, J	M, F	M	M	F, J
	Toe trembling	M	-	M	-	-
	Toe flagging	M, F	M, F	M	-	F
Body	Body lowering	M	M	M	-	
	Body jerking	M, F	M, F	M	M	F
	Running-jumping	M, F, J*	M, F	M	-	-
	Upright posture	M*, F	F	-	-	F

The characterization of the visual signals follows the descriptions presented by Hödl & Amézquita (2001) and by Hartmann et al. (2005a), and the new signal Both legs kicking is described in the results section. Abbreviations: M, male; F, female; J, juvenile.

∗ Social context not determined.

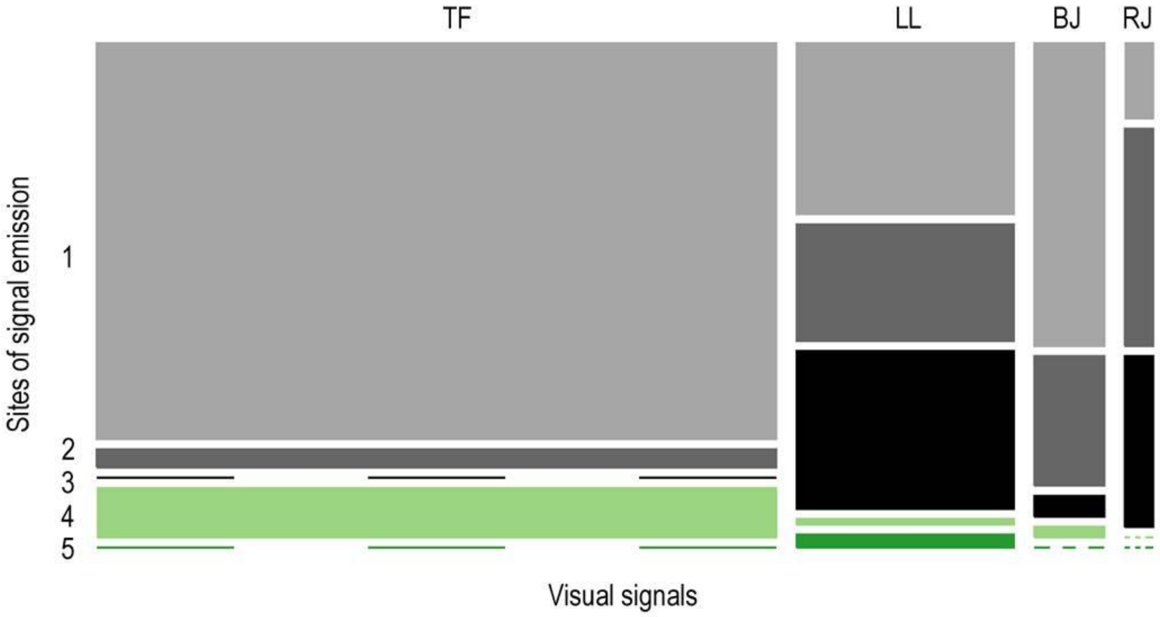

Figure 1. Mosaic distribution of the most frequent visual signals of *Crossodactylus schmidti* (674 signalizations from 54 individuals) according to the sites used for signal emission, based on 22 h of observations in Parque Estadual do Turvo, northwest of Rio Grande do Sul, Brazil. Each column represents the total frequency of emission of a visual signal and the numbered lines represent the emission sites used to signalize a visual signal; the relative frequency of signal emission is proportionally distributed within rectangles, and dashes represent the absence of a visual signal for the respective site. Abbreviations: TF, Toe flagging; LL, Limb lifting; BJ, Body jerking; RJ, Running-jumping; 1, on rock, above the water level; 2, on rock, at the water level; 3, on rock, below the water level; 4, on vegetation, at the water level; 5, on marginal vegetation. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x.

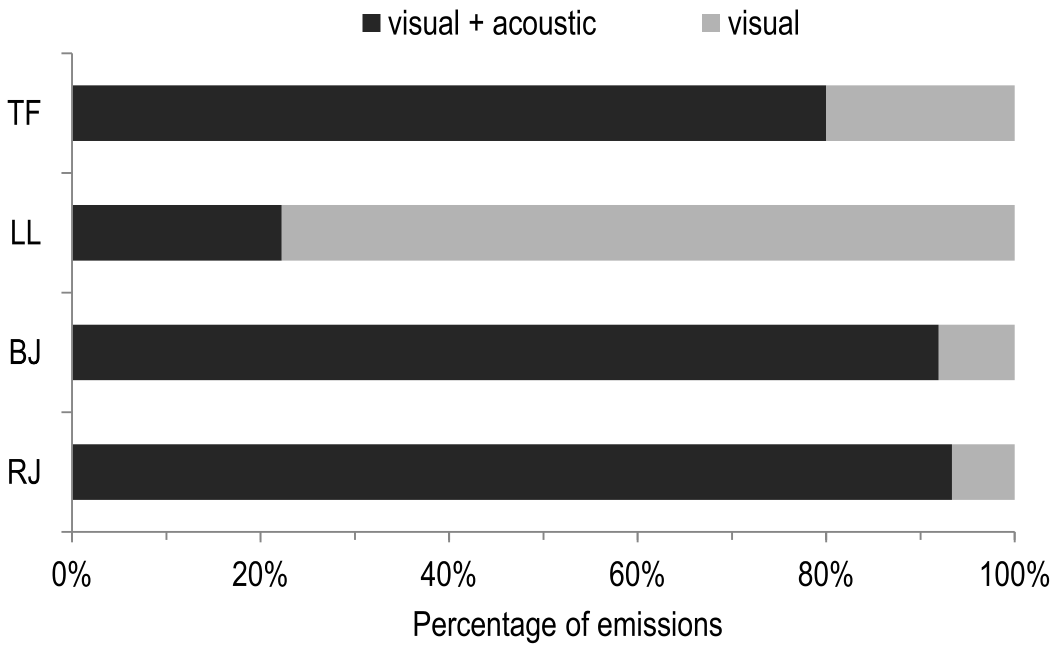

Figure 2. Percentage of unimodal (visual) and multimodal (visual + acoustic) displays emitted by males of *Crossodactylus schmidti* (227 signalizations) considering the most frequent visual signals, based on 6h of representative focal recordings of advertising males (*N* = 2), agonistic interactions between males (*N* = 22) and courtship interactions (*N* = 12), selected from the total time of recordings obtained in Parque Estadual do Turvo, northwest of Rio Grande do Sul, Brazil. Abbreviations: TF, Toe flagging; LL, Limb lifting; BJ, Body jerking; RJ, Running-jumping.

Table 2. Representative acoustic, visual and multimodal communication of males of *Crossodactylus schmidti* engaged in different social interactions at Parque Estadual do Turvo, northwest of Rio Grande do Sul, Brazil.

Advertisement display	Agonistic interaction between males		Courtship interaction
	Male 1	Male 2	
Adv	Agg + BJ *	Agg + BJ *	Agg
Adv + TF *	TF	Agg + BJ *	Adv → LL **
Agg	Agg + BJ *	Agg	Agg + Adv
Adv + TF *	TF	Agg + BJ *	Adv (28 x)
Agg + LK *	Agg + BJ *	Agg + TF *	LL
Agg + TF *	Agg + TF *	Agg + BJ	Adv (22 x)
Adv + TF *	Agg + TF *	Agg + TF *	Adv → LL **
LK	Agg + TF *	-	Adv (24 x)
LK	Agg + TF *	-	Agg + Adv
LL	Agg + TF *	-	Adv + LL *
LL	Agg	-	Adv (4 x)
Adv	Agg + TF *	-	Agg + Adv
BLK	Agg + TF *	-	Adv (47 x)
Adv	TF	-	Adv + RJ *
BLK	Agg + TF *	-	Adv + RJ *
Adv	Agg + TF *	-	Adv + RJ *
LL	Agg + TF *	-	Adv + RJ *
Adv	TF	-	Adv + RJ *
-	-	-	Adv (9 x)

The behaviours are listed in the same sequence in which they were performed by focal males during advertisement display on rock above the water level, lasting 5 min; agonistic interaction between two males, on rock above the water level, with duration of 10 min; and courtship interaction, duration of 70 min. Abbreviations: Adv, advertisement call; Agg, aggressive call; TF, Toe flagging; LL, Limb lifting; BJ, Body jerking; RJ, Running-jumping; LK, Leg kicking; BLK, Both legs kicking.

* Multimodal display with simultaneous components.

** Multimodal display with sequential components.

4. Discussion

4.1. Repertoire of visual signals

Crossodactylus schmidti presents a repertoire of visual signals consistent with the complexity of the visual repertoire of other diurnal stream frogs. Hylodidae species are diurnal and inhabit streams, attributes considered related to the evolution of visual communication (Hödl & Amézquita, 2001). Indeed, visual communication was observed for several species of *Hylodes* and at least for one species of *Crossodactylus*. Two signals emitted by *C. schmidti* are shared with *C. gaudichaudii*, i.e., Limb lifting and Body jerking (Weygoldt & Carvalho-e-Silva, 1992), and five signals with species of Hylodes, i.e., Limb lifting, Toe flagging, Toe trembling, Upright posture and Leg kicking (Haddad & Giaretta, 1999; Wogel et al., 2004; Hartmann et al., 2005a; Narvaes & Rodrigues, 2005; Lingnau et al., 2008; Forti & Castanho, 2012).

We demonstrated that males of *C. schmidti* used most of the visual signals during agonistic interactions, a result that draws attention to the role of these interactions on signal evolution. It has been suggested that signals as Leg kicking and Foot flagging may have evolved from the ritualization of aggressive movements performed during combats (Giasson & Haddad, 2006; Preininger et al., 2013). In this sense, the competitive pressure among males could be related to the convergent evolution of signals, such as the Foot flagging in *Hylodes* species of the Neotropics (e.g., Haddad & Giaretta, 1999), in *Staurois* species from Borneo (Grafe & Wanger, 2007) and in *Micrixalus saxicola* from India (Krishna & Krishna, 2006). A recent comparative study of the signals of *S. parvus* and *M. saxicola* showed that in the latter, the Foot flagging may have evolved from aggressive kicks and could represent an emerging evolutionary state in the ritualization of a visual signal (Preininger et al., 2013). In contrast, the absence of Foot flagging in species of *Crossodactylus* is interesting regarding the convergent evolution of behaviours. The absence of Foot flagging and the presence of aggressive signals characterized by rapid limb movements in *C. schmidti*, i.e., Leg kicking and *Both legs kicking*, may indicate that competitive pressure among males has favoured the evolution of rapid visual signals. Alternatively, the Leg kicking could represent the current state of a signal that, if selection occurs in this direction, could gradually increase its conspicuity.

Agonistic interactions between juveniles and adults of *C. schmidti* involved the use of visual signals by both groups. In such interactions adults chased and expelled juveniles, in contrast to that observed for *H. dactylocinus*, whose males apparently tolerate their presence

(Narvaes & Rodrigues, 2005). It is known that *C. schmidti* can actively forage aquatic and terrestrial prey and that the diet of adults and juveniles overlaps (Caldart et al., 2012). Therefore, since males of *Hylodes* and *Crossodactylus* species are territorial and often engage in agonistic interactions (Weygoldt & Carvalho-e-Silva, 1992; Haddad & Giaretta, 1999), it is reasonable to assume that the territorial behaviour of males may lead to agonistic interactions with juveniles during foraging activity. On the other hand, territorial behaviour in females of Hylodidae is unclear (but see Narvaes & Rodrigues, 2005), though the use of visual signals by *C. schmidti* females toward juveniles suggests that some degree of territoriality may lead to agonistic interactions.

Rocks are used as calling sites by diurnal stream frogs that utilize visual signals (Narvaes & Rodrigues, 2005; Krishna & Krishna, 2006; Grafe & Wanger, 2007; Caldart et al., 2010). *Crossodactylus schmidti* emitted most visual signals predominantly from rocks above the water level, which can be advantageous as it probably facilitates signal transmission and detection in a complex signalling environment. As visual signals may be degraded by a set of physical barriers that interferes on signal transmission (Higham & Hebets, 2013), the evolution of large visual repertoires in diurnal frogs inhabiting acoustically complex environments may be favoured by signaling from sites free of visual barriers. On the other hand, nocturnal species that signal from sites of high visual disturbance (e.g., amidst dense vegetation) may have small visual repertoires, as observed for *Dendropsophus parviceps* (Amézquita & Hödl, 2004). These findings suggest that in addition to the diurnal activity and presence in noisy habitats, the frequent agonistic interactions and the use of rocks for signal emission have also played a role in the evolution of the complex visual repertoire of *C. schmidti*.

4.2. Association between visual and acoustic signals

Some frogs emit multimodal displays characterized by the association of visual and acoustic signals (Grafe & Wanger, 2007; Preininger et al., 2009; Grafe et al., 2012). Although the function of multimodal displays remains unclear for most species, it is known that it may vary according to the type of selective pressures acting on signals (sensu Hebets & Papaj, 2005). For example, the advertisement call of *S. guttatus* may precede Foot-flags and have an alert function in intraspecific communication, by focusing the receiver’s attention to the subsequent visual signals (Grafe & Wanger, 2007). Visual and acoustic signals may also be synchronized and have the function of compensating the efficacy of both signals, as the conspicuous visual stimulus of the inflated vocal sac of males of *Physalaemus pustulosus* (Rosenthal et al., 2004).

In males of *C. schmidti*, three of the four main visual signals were emitted simultaneously with aggressive calls (i.e., Toe flagging, Body jerking and Running-jumping). This can be attributed to the frequent agonistic interactions between males and that males of *C. schmidti* can emit long aggressive calls during these interactions (Caldart et al., 2011). Thus, the longer the interaction, the longer the duration of the aggressive calls and the more frequent the emission of associated visual signals. On the other hand, the frequent emission of Limb lifting isolated from acoustic signals suggests that this signal is mainly employed in a unimodal way; however, the use of this signal in a multimodal context should not be ignored, since it was also emitted during, before or after calls. As the multimodal communication in males of *C. schmidti* seems to be quite complex, the association between visual and acoustic components may be more variable than described herein.

Multimodal displays are not exceptions in nature, but information about this type of communication in frogs is scarce, making it difficult to understand the function of these displays and the contribution of different types of pressures that could act on them. While such studies are scarce with frogs, our results indicate that *C. schmidti* represents an appropriate model for studies on the function of complex displays (sensu Hebets & Papaj, 2005) due to the presence of isolated visual and acoustic signals, as well as the use of audiovisual displays with synchronized or temporally-associated components.

4.3. Derived behaviours similar to visual signals

Classical ethologists discussed the phenomenon of the derived activities and provide the theoretical basis for the evolution of new behaviours through ritualization (Tinbergen, 1952; Huxley, 1966; Lorenz, 1966). Recent evidence supports the classical principles of the evolution of signals by means of ritualization (Scott et al., 2010), considered the main process by which animal signals evolve (Scott-Phillips et al., 2012). Despite of being frequent and susceptible to the ritualization process, descriptions of derived behaviours in frogs is unusual (but see Radcliffe et al., 1986). Regarding visual communication, distinctions between visual signals and similar derived behaviours could provide clues about what kind of non-communicative actions could be modified to the point of becoming ritualized signals. For example, the contralateral hind limb movements in *Atelopus zeteki* suggest that the signal Arm waving represents an elaboration of a standard stepping movement (Lindquist & Hetherington, 1998).

Sudden and repeated up-and-down movements of the limbs, similar to Limb lifting signal, were observed as derived behaviours in *C. schmidti* during ingestion of large prey, a behaviour also observed in males of *H. dactylocinus* in the same context (Narvaes & Rodrigues, 2005), and when frog-biting midges attacked a calling male. The interaction with midges, particularly, is very important as it draws attention to the possible influence of eavesdroppers on the evolution of visual signals in *C. schmidti*. Frog-biting midges (*Corethrella*) are known to parasitize male frogs worldwide locating them through phonotaxis, a relationship dating from the Early Cretaceous (Borkent, 2008; Borkent & Grafe, 2012). Some studies demonstrate distinct behavioural and evolutionary effects emergent from this interaction. Males of *Hyla avivoca* attacked by flies on the limbs performed repeated limb movements to displace them (McKeever, 1977), similar to the movements observed for *C. schmidti* in the same context. For the frog *P. pustulosus* it was shown that females flies (Bernal et al., 2006), as well as bat predators (Tuttle & Ryan, 1981) and conspecific females (Ryan, 1980) were more attracted by the complex calls of males, generating opposing pressures by natural and sexual selection on their acoustic signals. Furthermore, it was shown that females of *Corethrella* can transmit trypanosomes when obtaining a bloodmeal on frog calling males (Johnson et al., 1993; Ferguson & Smith, 2012).

Given the accumulation of observations of *Corethrella* attacking male frogs, Grafe & Wanger (2007) suggested that this interaction may constitute an additional pressure in the evolution of visual communication in frogs, and that males of *S. guttatus* may have reduced calling activity and increased the use of visual signals to avoid attacks of midges. Strengthening the general suggestion of Grafe & Wanger (2007), our observation provides circumstantial evidence that frog-biting midges may have exerted influence on the evolution of the visual repertoire of *C. schmidti*. As *C. schmidti* widely uses acoustic, visual and multimodal signals, we suggest that some visual signals, i.e., Limb lifting, may have first emerged as a behaviour to repel attacks of eavesdroppers like frog-biting midges, and may have been later co-opted for intraspecific communication. Derived movements performed by males in an attempt to repel the attacks of flies could inadvertently signal possession of territory to attentive conspecifics, and gradually evolve into a ritualized visual signal with a communicative function.

4.4. Concluding remarks

Communication in *C. schmidti* involves a large repertoire of visual and acoustic signals as well as multimodal displays. We suggest that in addition to historical factors related to the evolution of visual communication, such as diurnal activity and the presence in acoustically complex habitats, additional factors may have also contributed to the evolution of the complex visual repertoire of *C. schmidti*. These may include: (1) behavioural factors, such as (i) the use of sites free of visual barriers for signal emission, and (ii) the frequent and prolonged agonistic interactions between males and between adults and juveniles; and (2) ecological factors, such as the potential selective pressure on calling males imposed by frog-biting midges. Still, given the occurrence of various derived behaviours similar to visual signals, it is likely that the ritualization of these actions in the past may have led to the evolution of part of the visual repertoire.

This study aimed to describe the visual and multimodal communication in *C. schmidti* and to discuss possible causes and processes related to the evolution of its complex visual repertoire. Experimental investigations and comparative approaches on the function of the visual and multimodal displays of *C. schmidti* and other species of Hylodidae are necessary to test the hypothesis and ideas herein proposed. Moreover, it is necessary to further investigate the interaction between calling males and frog-biting midges in the southern neotropics through collections using acoustic traps with frog calls. Our study provides novel results on visual and multimodal communication for the genus *Crossodactylus*, which potentially contributes to the design of studies aiming to address specific questions about the visual and multimodal communication in frogs.

Supplementary video 1. Some visual signals and multimodal displays of *Crossodactylus schmidti*, recorded in Parque Estadual do Turvo, northwest of Rio Grande do Sul, Brazil.

Supplementary video 2. Part of the interaction between a calling male *Crossodactylus schmidti* and frog-biting midges, recorded on September 22, 2009 at 13.05 h, in Parque Estadual do Turvo, northwest of Rio Grande do Sul, Brazil.

Acknowledgements

We are grateful to Luciane A. Peres, Luís O.M. Giasson, Rodrigo Lingnau, Vanessa B. Fortes and two reviewers for their valuable suggestions that greatly improved the final version of the manuscript. We also thank Glauco Machado for kindly discussing some of the recorded behaviours, and Victor M. Lipinski, Bruno Madalozzo and Tiago R.N. Bertaso for their assistance in field activities. V.M.C. and S.I. thank CAPES for the doctoral fellowships, and S.Z.C. is grateful to CNPq for the award of a research fellowship (No. 304929/2012-3). Activities in PET were performed in accordance with the access licenses (Nos 133/2010 and 23/2011) issued by SEMA-RS.

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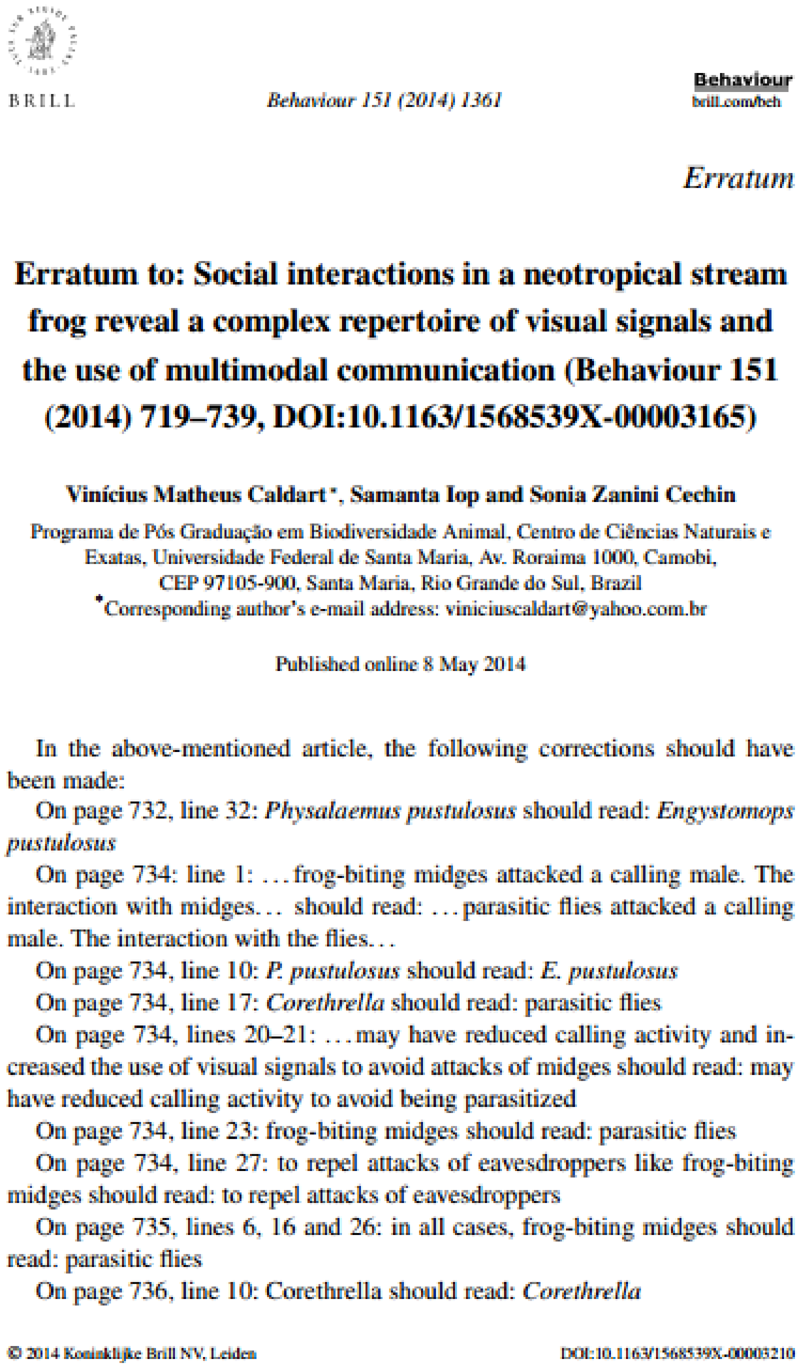
Anexo 1. Errata publicada pela editora Brill, referente às correções textuais solicitadas na etapa de correção da prova do manuscrito.



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Erratum

Erratum to: Social interactions in a neotropical stream frog reveal a complex repertoire of visual signals and the use of multimodal communication (Behaviour 151 (2014) 719–739, DOI:10.1163/1568539X-00003165)

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In the above-mentioned article, the following corrections should have been made:

On page 732, line 32: *Physalaemus pustulosus* should read: *Engystomops pustulosus*

On page 734: line 1: . . . frog-biting midges attacked a calling male. The interaction with midges. . . should read: . . . parasitic flies attacked a calling male. The interaction with the flies. . .

On page 734, line 10: *P. pustulosus* should read: *E. pustulosus*

On page 734, line 17: *Corethrella* should read: parasitic flies

On page 734, lines 20–21: . . . may have reduced calling activity and increased the use of visual signals to avoid attacks of midges should read: may have reduced calling activity to avoid being parasitized

On page 734, line 23: frog-biting midges should read: parasitic flies

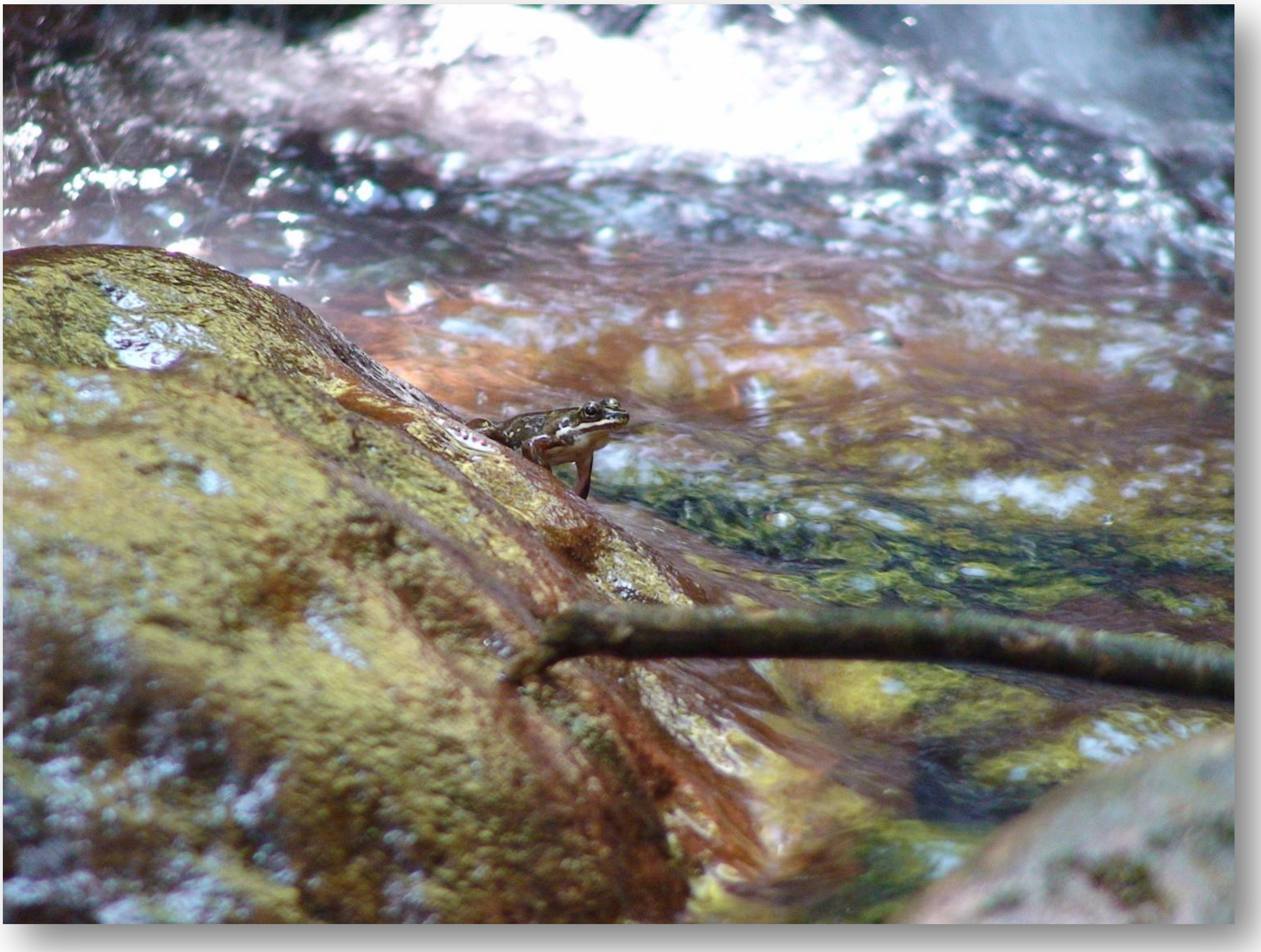
On page 734, line 27: to repel attacks of eavesdroppers like frog-biting midges should read: to repel attacks of eavesdroppers

On page 735, lines 6, 16 and 26: in all cases, frog-biting midges should read: parasitic flies

On page 736, line 10: Corethrella should read: *Corethrella*

DOI:10.1163/1568539X-00003210

CAPÍTULO III



Acoustic communication in a Neotropical diurnal stream frog (Anura, Hylodidae): temporal patterns of calling and noise-dependent adjustments of call traits

Acoustic communication in a Neotropical diurnal stream frog (Anura, Hylodidae): temporal patterns of calling and noise-dependent adjustments of call traits

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Abstract. Acoustically active animals have evolved adaptations and may employ shifts in acoustic traits to cope with ambient noise. However, disentangle long- and short-term adaptations related with noise is not trivial and this topic has been little addressed for Neotropical diurnal stream frogs. Here we investigated the role of environmental factors on the temporal patterns of calling activity of the diurnal stream frog *Crossodactylus schmidti*, and the signal-to-noise relations between its advertisement call and the ambient noise. Calling males occurred year-round, with variable effects of environmental factors: monthly activity increased with accumulated rainfall and air temperature, early morning activity increased with air temperature, and the daytime activity increased with light intensity. Calls greatly varied in spectral structure, with variable location of note dominant frequency (NDF) among harmonics. We found a pattern of overlapping between signal and noise frequencies with noise filtered at the NDF. Call and noise frequencies did not differ with noise filtered at the 1st or 3rd harmonic, but differed at the 2nd. NDF had a mean increase of 300 Hz from the 1st to the 15th call note. Call SPL exceed noise SPL at the 1st and 2nd harmonics in a mean difference of 5 dB, but did not differ at the 3rd. Yet, all males emitted notes with negative signal-to-noise ratios regardless of the harmonic with the NDF. We concluded that males of *C. schmidti* call year-round, that the multi-note harmonic call and the use of visual/audiovisual signals may have evolved due to the masking effect of the stream noise on acoustic signals, whilst the NDF modulation with variable location among harmonics and the increased NDF along notes are behavioral shifts related with temporary, subtle changes in stream noise. This study uncouples long- and short-term adaptations for a Hylodidae species for the first time, providing insights into signal evolution and behavioral plasticity of diurnal stream frogs.

Keywords: *Crossodactylus*, calling activity, environmental factors, signal-to-noise ratio, acoustic adaptation, acoustic plasticity

1. Introduction

Acoustic communication has a central role in the life history of anurans as it mediates social interactions (Wells 1977; Hutter et al. 2013), increases risk of predation (Tuttle and Ryan 1981; Halfwerk et al. 2014) and parasitism (Bernal et al. 2006; Ferguson and Smith 2012), is directly linked to breeding activity and ultimately to reproductive success (Gerhardt 1991; Tárano and Herrera 2003; Tárano and Fuenmayor 2013). Calling activity may be affected by intrinsic factors, such as ontogeny and circadian rhythm (Oishi et al. 2004), and by extrinsic factors which may include a set of environmental conditions (Wells 2007). The combination of intrinsic and extrinsic factors will influence the temporal strategy of reproduction, which may range from sporadic and explosive events of breeding activity, often triggered by a specific climatic factor, e.g. heavy rains, to prolonged and continuous patterns of reproduction, when calling activity occurs for several months or throughout the year (*sensu* Crump 1974; Wells 1977; Wells 2007).

The role of environmental factors on the temporal patterns of calling activity is relatively well known for tropical and subtropical anuran communities from the Neotropical region, where most species are predominantly nocturnal and inhabit lentic habitats (e.g., Both et al. 2008; Zina et al. 2007; Van Sluys et al. 2012). Although it is known that environmental factors may affect different species in different ways within a given community (Ospina et al. 2013), the calling activity of tropical anuran communities is in general strongly affected by variation in air temperature and rainfall (Zina et al. 2007; Van Sluys et al. 2012), whereas in subtropical communities the activity is mainly affected by variation in air temperature and photoperiod (Both et al. 2008; Canavero et al. 2008; Canavero and Arim 2009). Although this is a consistent scenario for most nocturnal communities, much less is known about the effects of environmental factors on the calling activity of diurnal frogs which inhabit streams – such as the frogs of the genera *Hylodes* and *Crossodactylus* (Hylodidae) from the Brazilian Atlantic Rainforest – making our understanding on temporal patterns of activity still incipient for this remarkable group of frogs.

To date, just a couple of studies with species of *Hylodes* and *Crossodactylus* from tropical areas of Brazil have investigated the effects of environmental factors on calling activity, indicating air temperature, photoperiod and rainfall as regulators of monthly variation of calling activity (Hatano et al. 2002), and air temperature and light intensity affecting the daily variation on calling activity (Almeida-Gomes et al. 2007). However, none studies have yet investigated the effects of environmental factors on calling activity of subtropical species of Hylodidae. With 46 species currently recognized in Hylodidae, the vast majority of them occur in tropical areas of the Brazilian Atlantic Rainforest (Frost 2015; Segalla et al. 2014), where species of *Hylodes* and *Crossodactylus* may even co-occur (Almeida-Gomes et al. 2007a), whereas just a few species have a subtropical distribution reaching the southernmost portion of Brazil (i.e. *Crossodactylus schmidti*, Caldart et al. 2013; *Hylodes meridionalis*, Lingnau et al. 2013).

Frogs living alongside and within streams usually have to deal with ecological problems inherent of lotic habitats to properly communicate. Given that in their habitats the noise generated by other frog species is low or absent – in comparison with the noisy choruses of several species breeding in ponds – abiotic noise often constitutes a major source of acoustic interference in streams. To reduce the effect of background noise in signal transmission and perception – in the perspectives of both senders and receivers – it has been showed that animals have evolved different behaviors that increase the signal-to-noise ratio (Endler 1992; Endler 1993). Hence, as directly linked with breeding activity and reproductive success, anuran advertisement calls are expected to have evolved to increase their signal-to-noise ratio in noisy habitats. For example, as an adaptive response to the historical constant ambient noise, stream breeders may have evolved high frequency calls which greatly contrast against the low frequency stream-generated noise (Grafe et al. 2012; Preininger et al. 2013a) and/or may have exploited different sensory modalities to communicate, e.g. visual signaling (Hödl and Amézquita 2001; Hartmann et al. 2005; long-term adaptations *sensu* Brumm & Slabbekoorn 2005). On the other hand, short-term adjustments in acoustic traits may also be used as strategies to cope with temporary or subtle changes in ambient noise, which may include regulation of signal frequency, amplitude, redundancy and duration (short-term adaptations *sensu* Brumm and Slabbekoorn 2005). For instance, a widespread short-term strategy used by mammals and birds to cope with acoustic interference is to regulate call amplitude as the level of ambient noise increases (i.e. the Lombard Effect, Brumm and Zollinger 2011). To what extent this strategy is used by anurans has been a matter of debate, but an experimental test showed that males of the grey treefrogs (*Hyla chrysoscelis*) are unable to regulate call amplitude as a function of habitat noise (Love and Bee 2010); instead, the tested frogs showed adjustments in call duration and call rate to cope with noise. Accordingly, a field experiment found that males of green frogs (*Rana clamitans*) and leopard frogs (*Rana pipiens*) increased call frequency in response to traffic noise, but did not increase call amplitude (Cunnington and Fahrig 2010), suggesting that noise-dependent regulation in anurans must be more common on call traits other than signal amplitude.

Diurnal stream frogs have been shown to have remarkable adaptations to communicate. In contrast with many anuran taxa, species the Neotropical genera *Hylodes* have harmonic-structured calls with the dominant frequency located at the higher harmonics instead at the fundamental, which is considered a long-term adaptation for coping with the continuous but variable low frequency noise of streams (Vielliard and Cardoso 1996; Haddad and Giaretta 1999; Lingnau and Bastos 2007). This adaptation was also suggested for the close-related genus *Crossodactylus* (Pimenta et al. 2008; Caldart et al. 2011), whose species also inhabit streams and use visual and multimodal signals (Caldart et al. 2014). *Hylodes* and *Crossodactylus* are Neotropical taxa of interest in the study of anuran communication (Hödl and Amézquita 2001) because they widely use acoustic and visual signals (Haddad and Giaretta 1999; Caldart et al. 2014), but few studies have measured abiotic noise to investigate behavioral plasticity in call traits, despite the relevance of ambient sound level to characterize anuran habitat (Goutte et al. 2013; see Lingnau and Bastos, 2007). Hence, the data available to date point out for two major long-term adaptations in the communication of *Hylodes* and *Crossodactylus* – *i)* harmonic-structured calls with the dominant frequency at higher frequencies and *ii)* visual signaling –, while short-term adaptations to cope with noise have been much less investigated.

Here we investigated, under natural conditions, the following questions about the acoustic communication of the diurnal stream frog *Crossodactylus schmidti*: *1)* how is the males' calling activity distributed along the daily and monthly scales?; *2)* what is the role of environmental factors on the regulation of calling activity at both temporal scales?; *3)* do males adjust acoustic traits of advertisement calls according to the ambient noise produced by flowing water of streams? For the first two questions we predicted that: *i)* the daytime calling activity would be affected by light intensity, given that for a taxon which widely uses acoustic signals and associated visual displays, an increase in light intensity at calling sites would provide a better discrimination of the sender and its signals to intended receivers (Endler 1992; Endler 1993; Grafe and Wanger 2007), and that *ii)* the monthly calling activity would be affected by variation in air temperature and photoperiod, factors often related with the calling activity of subtropical frogs (Both et al. 2008; Canavero et al. 2008; Canavero and Arim 2009). Finally, for the later question we investigated noise-dependent regulation on acoustic signal traits by describing the acoustic signal and ambient noise structure. Given that acoustic signals have a key role in the life history of frogs and that habitat noise is a strong selective pressure in the communication of acoustically active animals, we expect to reveal both long-term and short-term adaptations in the communication of *C. schmidti.* Disentangle long and short-term acoustic adaptations within a communication system is a difficult task and at our knowledge this was never addressed for a Hylodidae species. Here we provide a first fine-scale investigation on this topic for this taxon.

2. Material and Methods

2.1. Study area

The study was conducted at the Turvo State Park (27°14'34.08"S, 53°57'13.74" W, 376 m.a.s.l.), located in the municipality of Derrubadas, northwest region of the state of Rio Grande do Sul, Brazil. The park is considered one of the last large remnants of mesophytic semideciduous forest (*sensu* Oliveira-Filho et al. 2006) in southern Brazil, covering an area of 17,491.4 ha (SEMA 2005) adjacent to the border of the Uruguay River and near the Argentinean forests of the Moconá Provincial Park and the Yabotí International Biosphere Reserve. The local climate is characterized as subtropical sub-humid with dry summer, with an annual rainfall of 1,787 mm, a mean annual temperature of 18.8 ºC and a mean temperature of the coldest month of 13.3 °C (ST SB v climate type, *sensu* Maluf 2000).

2.2. Data acquisition

2.2.1. Temporal patterns of calling activity

We investigated the temporal patterns of calling activity of a *C. schmidti* population through the method of acoustic monitoring within fixed points (Rand and Drewry 2001), in a section of 150 m along a first order stream (27°14'36.56"S, 53°57'14.30"W) in which we had confirmed the occurrence of an abundant population of *C. schmidti* (Iop et al. 2011; Caldart et al. 2012). We established three fixed sites for acoustic monitoring (Rand and Drewry 2001) in this section of the stream, apart at least 40 m from each other to avoid acoustic interference. From October 2011 to September 2012, two days per month and at hourly intervals during the daytime (from 05:00 to 19:00), the first author counted for ten minutes at each site the *1)* number of males in calling activity and the *2)* number of advertisement and aggressive calls emitted by all males. The procedure was employed always at the stream margin, considering only individuals calling within a perimeter of approximately five meters around the sites. To evaluate the occurrence of nocturnal activity, the same procedure was employed from

November 2011 to February 2012, one day per month and at hourly intervals during the night time (20:00 to 04:00). During each counting we measured the following variables at the sites with a THAL-300 (Instruterm®) device: air temperature, water temperature, air humidity and light intensity. Subsequently, we obtained for each day of monitoring the duration of the photoperiod (in minutes) from the Observatório Nacional Brasileiro (2014, http:/euler.on.br/ephemeris/index.php/), and the accumulated amount of rainfall for seven and fifteen days prior to each sampling, obtained from the database of INMET – Instituto Nacional De Meteorologia (2014, http://www.inmet.gov.br/) for the nearest weather station at the city of Iraí, Rio Grande do Sul, located 56 km from the study area.

The calling activity of *C. schmidti* was expressed as two dependent variables: *1)* the mean number of calling males per hour and month intervals, and *2)* the mean number of calls emitted per hour and month intervals (e.g., Heyer et al. 1994; Hatano et al. 2002; Almeida-gomes et al. 2007). Since we did two monitoring periods each month with the same population, we use only the maximum abundance of calling males and the maximum number of calls registered for each hour interval. Furthermore, as the monitoring sites were acoustically independent from each other, we summed the number of calling males and the number of calls of the three sites for each hour interval. Therefore, the mean activity for each hour interval was calculated upon 12 observations (twelve observations for each hour, i.e., twelve months), and the mean activity for each month interval was obtained upon 15 observations (fifteen observations for each month, i.e., hourly intervals between 05:00 to 19:00).

2.2.2. Call structure and signal-to-noise relations

We investigated noise-dependent regulation in acoustic traits by measuring peak sound pressure levels (SPL in dB) of calls and habitat noise in the field, with a sound level meter (Impac®, model IP-120C) at a distance of about 50 cm of individuals, and by a subsequent analysis of spectral and temporal traits of the recordings that served for the description of the species vocalizations (Caldart et al. 2011) and additional calls recorded on September 2013. Vocalizations were recorded at a distance of about 50 cm from the individuals, with a Marantz® PMD671 digital recorder coupled to a Sennheiser® ME66 directional microphone. Call structure was described according to the terminologies presented in Bosch and De la Riva (2004) and Kaefer and Lima (2012) for the following traits: notes per call (NC) is the total number of notes in a call; call duration (CD) is the duration of the call in seconds; number of notes per second (N/s) is the number of notes of the call divided by the call duration; note dominant frequency (NDF) is the frequency in Hertz at the peak amplitude of the note; note frequency modulation (NFM) is the difference in Hertz between the highest and the lowest dominant frequency of the call (equivalent as “call frequency modulation”); frequency bandwidth of harmonics 1, 2 and 3 (FBH1, FBH2, FBH3) is the difference in Hertz between the highest and the lowest dominant frequency calculated from values of NDF for each harmonic; and note amplitude (Note SPL) is the peak amplitude of the note in dB.

Given the harmonic structure of the calls and the variable location of the dominant frequency among harmonics, we used the information present in the species call description (Caldart et al. 2011) to apply a band-pass filter in the recordings, in order to obtain values of dominant frequency and SPL of notes and ambient noise at the range of the harmonics containing the NDF (Figure 1). This procedure is important because the level of noise interference will depend on the degree to which signal and noise frequencies overlap (Brumm and Slabbekoorn 2005). Thus, for each multi-note call we tracked the dominant frequency along 15 notes – 5 notes at the beginning, 5 at the middle and 5 at the end of the call – to extract at the NDF range spectral information of the acoustic signal and the ambient noise. Spectral measurements of ambient noise were obtained at the non-voiced gap prior to each note. The extracted relative sound pressure (SP) values of notes and noise, calculated through a FFT series in the bioacoustics’ software, were transformed into absolute SP (in dB, re 20 µPa) by the following equation: SP absolute = SP relative × SP measured/SP most intensive; where “SP most intensive” is the maximum SP of the complete call and “SP measured” corresponds to the maximum sound pressure recorded in the field (Boeckle et al. 2009; Grafe et al. 2012; Preininger et al. 2013a). Traits such as CD, NC and N/s were obtained from entire calls. Only advertisement calls were considered for analysis, because aggressive calls are much more variable in duration due to prolonged, escalated agonistic interactions between males (Caldart et al. 2011). Thus, variation in aggressive call traits may be more dependent on social interaction than on ambient noise interference. Yet, males may emit aggressive and advertisement calls in sequence (Caldart et al. 2011), and for such vocalizations we considered only the later calls for analysis.

Acoustic analyses were made in SoundRuler software v. 0.9.6.0 (http://soundruler.sourceforge.net; Bee 2004; Gridi-Papp 2004) at a sampling frequency of 44100 Hz and a resolution of 16 bits. Oscillograms and audiospectrograms were produced in R software v. 2.12.2 (R Development Core Team 2011) through the package TuneR (Ligges et al. 2013) and Seewave (Sueur et al. 2008) with the following parameters: Fast Fourier Transformation (FFT) method with 256 points, 100% frame, Hanning window type and 90% overlap. In total we analyzed 25 calls from ten males (mean snout-vent length was 27.2 mm ± 1.5), comprising 357 individual notes from 75 sections of five sequential notes. Means ± standard deviation and ranges, when applicable, are given as descriptive statistics.

2.3. *Statistical analysis*

2.3.1. Temporal patterns of calling activity

To evaluate the effect of the environmental variables on the daily and monthly calling activity, we used Generalized Regression Models (Nelder and Wedderburn 1972; Mccullagh and Nelder 1983) with forward stepwise model building (Zar 1999) in the software Statistica 10 (Statsoft 2011). To investigate the variables affecting the monthly calling activity we build a model for each dependent variable, including the following predictor variables: air temperature, air humidity, light intensity, photoperiod, seven days of accumulated rainfall and fifteen days of accumulated rainfall. To investigate variables affecting the daily calling activity, we first build a model for each dependent variable for the whole daytime interval (05:00 to 19:00), including the following predictor variables: air temperature, air humidity and light intensity. This procedure failed to detect a relationship between the predictor variables and the number of calling males and the number of calls, i.e. none variable was retained in the models ($P > 0.05$ for all predictor variables, see results), most likely because the early daily activity of *C. schmidti* (i.e. 05:00 and 06:00), as well as the values of the predictor variables for that hours (e.g. light intensity, see results), greatly differ from the rest of day time. Hence, given such differences, we analyzed the species early morning activity (05:00 to 06:00) and the remaining daytime activity (07:00 to 19:00) separately. Water temperature was not use in any models because it was strongly correlated with air temperature ($r > 0.8$ in all cases), and air humidity was excluded from models of early morning activity due to a strong correlation with air temperature for that hours ($r > 0.9$ in all cases). All variables were log transformed prior to analyses for statistical standardization, with the exception of air humidity, which was arcsine transformed.

We also investigated if the photoperiod affected the extension of the calling activity throughout the year, through a linear regression between the duration of photoperiod (in minutes) and the duration of the calling activity, obtained as the interval (in minutes) between the first and the last call recorded for the daily interval. Finally, we performed a circular statistical analysis (Zar 1999) in the software Oriana 4 (Kovach 2011), to investigate the distribution of the calling activity throughout the year, i.e., the occurrence or absence of seasonality in calling activity. Each month was converted in angles of 30º and the number of active males and calls were assumed as frequencies for each angle. We estimated the mean vector (μ), which represents the mean interval at which most of activity is concentrated; the circular standard deviation (*SD*) related to the mean vector; and the length of mean vector (r), a measure of data concentration around the circle (the year) which varies from 0 (dispersed data) to 1 (concentration of data in a particular direction). The Rayleigh Test of Uniformity (Zar 1999) was used to calculate the probability of the null hypothesis that the data are uniformly distributed throughout the year. A significant result of the Rayleigh test ($P < 0.05$) indicates that the data are not uniformly distributed and there is a significant mean direction, i.e., there is seasonality in calling activity.

2.3.2. *Call structure and signal-to-noise relations*

Linear regressions were used to test if males increased call SPL with increasing ambient noise, and to test if spectral and temporal traits of calls increased with note number. To test for differences in the dominant frequency and SPL between calls and ambient noise, we used Mann-Whitney pairwise post-hoc comparisons with Bonferroni corrected probability values, based on measurements of calls and noise, obtained after applying the band-pass filter at the range of the harmonic containing the NDF. To test for correlations between temporal call traits and noise SPL we used Pearson correlations (Zar 1999).

Finally, we obtained the signal-to-noise ratio (SNR) for notes and noise at call dominant frequency, by calculating the difference between the absolute SPL of individual notes and that of the ambient noise measured prior to each note. Values of SNR > 0 (in dB) mean that the acoustic signal has a positive signal-to-noise ratio, whereas values ≤ 0 mean that the ambient noise was louder than the acoustic signal. All data were tested for normality and for homogeneity of variances before performing statistical analyses, and were log-transformed if necessary; data used for parametric tests fitted a normal distribution. Analyses were made in the software Past version 3.04 (Hammer et al. 2001). Descriptive statistics are given as mean ± standard deviation, followed by range when applicable.

3. Results

3.1. Temporal patterns of calling activity

Males of *Crossodactylus schmidti* were active throughout the year, with a mean number of calling males variying from 2 ± 2 (range 0–3 males) in July, to 5 ± 2 (range 0–9 males) in October, November and March. In turn, mean number of emitted calls varied from 3 ± 2 (range 0–7 calls) in July, to 21 ± 17 (range 2–65 calls) in March, and 20 ± 16 (range 6–54 calls) in August (Figure 2, Figure S1). Although the reduction in calling activity in the coldest months, there was no seasonality in the number of calling males ($\mu = 329.117°$, SD = 122.034°, r = 0.103; Rayleigh Test = 0.471, $P = 0.627$), neither in the number of calls emitted ($\mu = 339.102°$, SD = 117.782°, r = 0.121; Rayleigh Test = 2.411, $P = 0.090$). Similarly, the variation in photoperiod did not affect the extension of the calling activity throughout the year ($R^2_{adj} = 0.06$, $F = 0.602$, $P = 0.456$).

Crossodactylus schmidti presented a predominantly diurnal calling activity (Figure 2, Figure S1), with a prevalent and pronounced peak in the early morning occurring both in the number of calling males (*05:00*, mean = 6 ± 2, range 0–8 males; *06:00*, mean = 5 ± 2, range 1–9 males) and in the number of calls (*05:00*, mean = 25 ± 16, range 0–48 calls; *06:00*, mean = 26 ± 21, range 1–65 calls), followed by a pattern of increasing activity along the daytime hours, and then by a decrease in activity at dusk. On the opposite, nocturnal activity was sporadic, with a few number of calling males (mean = 1 ± 1, range 0–4 males) and number of calls (mean = 1 ± 2, range 0–17 calls).

Environmental factors had significant positive effects on the calling activity of *C. schmidti*, though have affected it in different ways (Table 1). The monthly number of calling males increased with accumulated rainfall – which was also marginally affected by air temperature – while the monthly number of emitted calls increased with accumulated rainfall and air temperature (Figures S2-S5). Regarding the early morning activity (05:00 to 06:00), both number of calling males and number of emitted calls increased with air temperature (Figures S6-S8), whilst during the day time hours (07:00 to 19:00) both number of calling males and emitted calls increased with light intensity (Figure S9-S11).

3.2. Call structure and signal-to-noise relations

The advertisement call of *Crossodactylus schmidti* showed noticeable variation at the spectral structure (Table 2). Note dominant frequency was usually located at frequencies around 3244 Hz, a range which encompasses both the first and second harmonics and, sometimes, the third harmonic. Most of the notes (269; 75%) had the dominant frequency located at the second harmonic – which had the widest bandwidth, ranging from 2245 to 4315 Hz – although in some notes it was at the first (61; 17%) or third harmonic (27; 8%). Thus, frequency modulation greatly varied among calls, and when the frequency modulation of a call was high (e.g. >1000 Hz), we could clearly attribute it to a change in the location of the harmonic containing the dominant frequency.

The investigation at the habitat with a sound level meter showed that males (n = 20) increased call SPL with increasing ambient noise ($R^2_{adj} = 0.93$, $F = 280.2$, $P < 0.001$), in a mean difference of 3 dB (± 3 dB) between signal (66 dB ± 6 dB) and noise (63 dB ± 7 dB); however, such difference do not necessarily reflect a real difference between signal and noise SPL, because the peak SPL of habitat noise as measured by the sound level meter may not be within the range of the dominant frequency of the call. Therefore, after applying filters in noise at the range of call harmonics, we found that call SPL significantly exceed the SPL of noise at the first harmonic (call = 73 ± 3, range 67-77 dB; noise = 68 ± 4, range 59-75 dB) and at second harmonic (call = 73 ± 2, range 63-77 dB; noise = 68 ± 4, range 58-76 dB), with a mean difference of 5 dB between signal and noise at the mean frequencies of the harmonics. On the other hand, the SPL of acoustic signal and the ambient noise did not differ significantly at the third harmonic (call = 72 ± 3, range 63-77 dB; noise = 70 ± 4, range 58-75 dB) (Figure 3).

Regarding the frequency domain, in turn, we found a pattern of great overlapping between signal and noise dominant frequencies with the ambient noise filtered at the range of call harmonics (Figure 4). Hence, call and noise dominant frequencies did not differ with noise filtered neither at the first (call = 2592 ± 311, range 2186-3109 Hz; noise = 2559 ± 295, range 2115-3310 Hz) nor at the third harmonics (call = 4034 ± 366, range 3706-5172 Hz; noise = 3619 ± 1035, range 2021-5290 Hz), though call and noise dominant frequencies significantly differed with noise filtered at the second harmonic (call = 3312 ± 366, range 2245-4315 Hz; noise = 3138 ± 611, range 2015-5456 Hz) (Figure 3). Overall, both the dominant frequency and the peak SPL varied much more for the ambient noise than for the acoustic signal.

Remarkably, we found that all males emitted notes presenting negative signal-to-noise ratios, with a mean of 3 notes per call (± 2, range 0-7 notes) below ambient noise SPL at call dominant frequency. In total, 22% of analyzed notes (77 notes) had a negative signal-to-noise ratio with noise filtered at call dominant frequency at the first, second or third harmonics (Figure 5). With respect to variation in acoustic traits along the call, note frequency increased with note number (Figure 6), with a mean increase of 300 Hz from the 1st to the 15th note (frequency – note number: $R^2_{adj} = 0.55$, $F_{1,13} = 18.48$; $P = 0.001$). Differently, note amplitude and note duration did not increase with note number (amplitude – note number: $R^2_{adj} = 0.05$, $F_{1,13} = 1.81$; duration – note number: $R^2_{adj} = 0.07$, $F_{1,13} = 2.02$; $P > 0.05$ for both tests). Regarding regulation on temporal call traits, call duration was strongly correlated with number of notes per call ($r = 0.82$, $P < 0.001$), but number of notes per call was not correlated with noise SPL at call frequency ($r = 0.19$, $P > 0.05$). Likewise, number of notes per second was not correlated with noise SPL at call frequency ($r = 0.08$, $P > 0.05$).

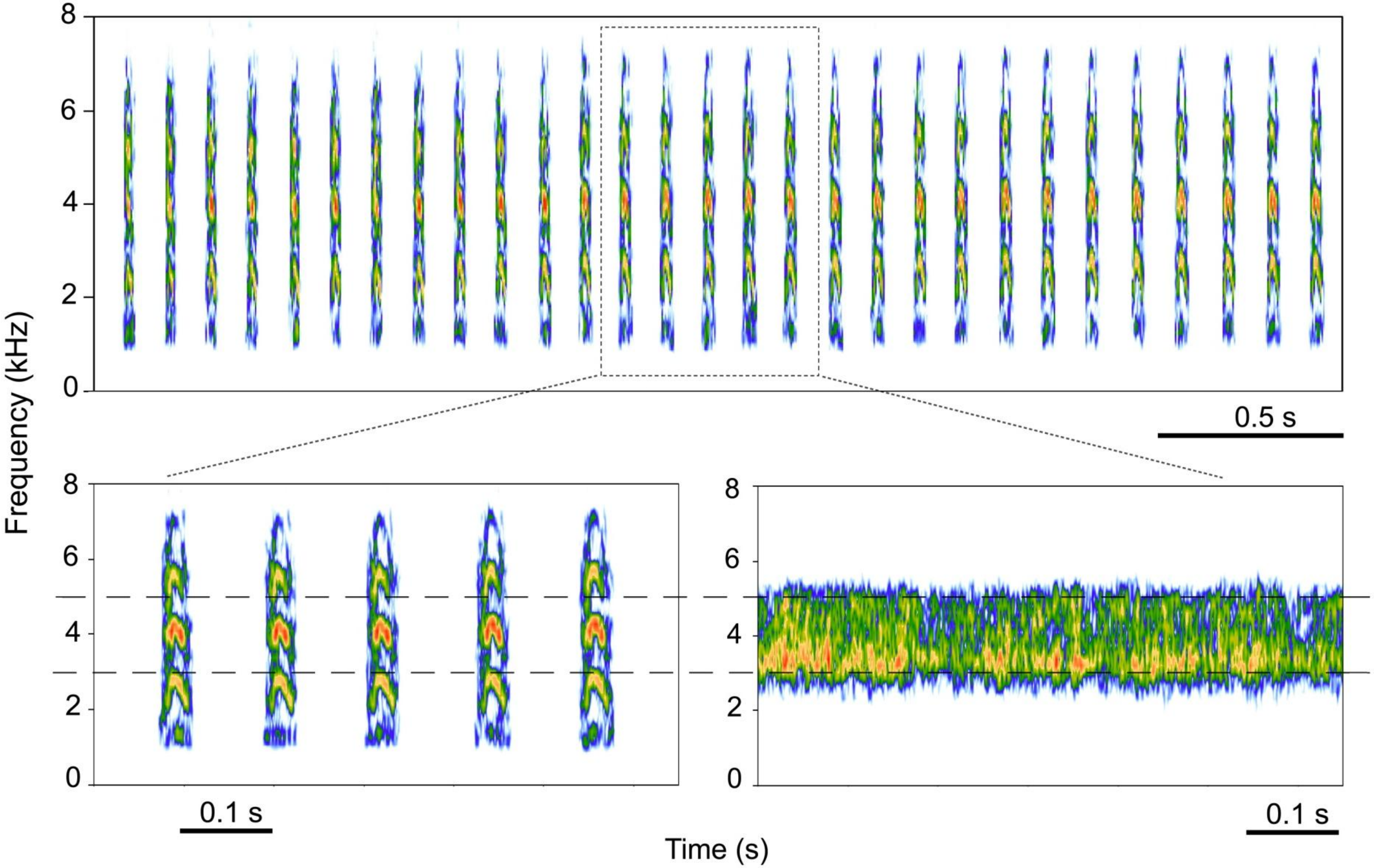

Figure 1. Advertisement call of the diurnal stream frog *Crossodactylus schmidti* (upper spectrogram), five notes extracted at the middle of the call (bottom left spectrogram), and the ambient noise filtered at the range of the dominant frequency of the notes (bottom right spectrogram). As shown in this call, note dominant frequency is usually located in the second harmonic, although it may also be located in the first or in the third harmonics.

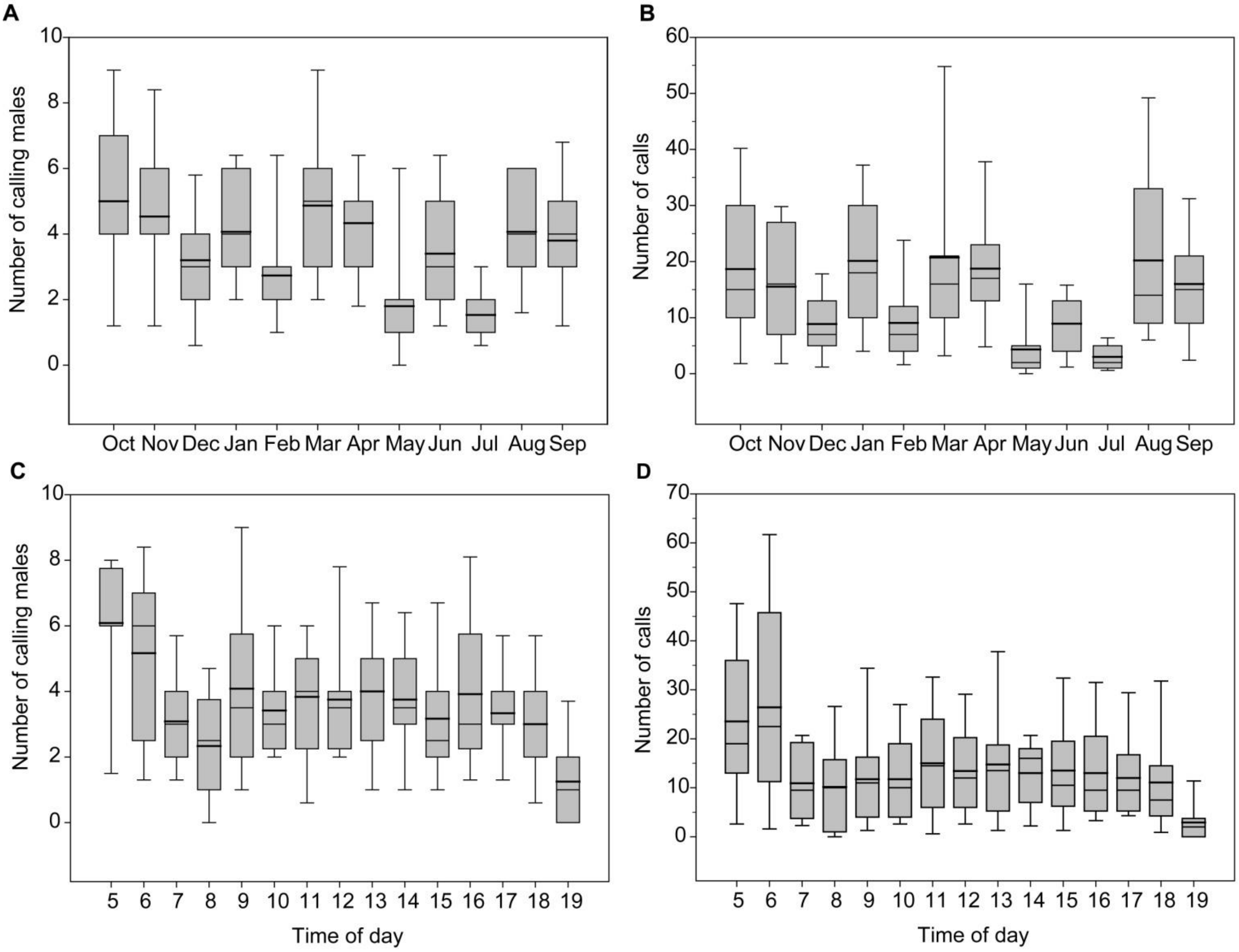

Figure 2. Temporal patterns of calling activity of the diurnal stream frog *Crossodactylus schmidti* in southern Brazil. A) Number of calling males at monthly intervals; B) number of calls registered at monthly intervals; C) number of calling males at hourly intervals; D) number of calls registered at hourly intervals. Box plots show the median (thinner solid line) and mean (tick solid line) values with interquartile range and 10th and 90th percentile.

Table 1. Results of the generalized regression model analysis of the effects of environmental factors on the temporal calling activity of the diurnal stream frog *Crossodactylus schmidti* in southern Brazil. Environmental factors in bold were retained in the final models, with a statiscally significant value (*P* <0.05).

Model	Effect	Number of calling males					Number of calls				
		ß	*F*	*d.f.*	*P*	R^2_{adj}	ß	*F*	*d.f.*	*P*	R^2_{adj}
Monthly activity			14.90	1, 10	**0.003**	**0.56**		14.12	2, 9	**0.002**	**0.70**
	15 days accumulated rainfall	0.77	14.90		**0.003**		0.59	12.36		**0.007**	
	7 days accumulated rainfall	-0.02	0.00		0.966		0.15	0.25		0.633	
	Air temperature	0.38	4.83		0.056*		0.52	9.67		**0.013**	
	Air humidity	-0.07	0.11		0.751		-0.11	0.36		0.565	
	Light intensity	0.22	0.91		0.365		0.18	0.65		0.445	
	Photoperiod	0.33	3.17		0.109		0.16	0.80		0.397	
Early morning activity (5:00 to 6:00)			17.73	1, 22	**<0.001**	**0.42**		23.04	1, 22	**<0.0001**	**0.49**
	Air temperature	0.67	17.73		**<0.001**		0.72	23.04		**<0.0001**	
	Light intensity	-0.17	1.21		0.284		-0.16	1.11		0.304	
Daytime activity (7:00 to 19:00)			25.63	1, 11	**<0.001**	**0.67**		31.32	1, 11	**<0.001**	**0.72**
	Light intensity	0.84	25.63		**<0.001**		0.86	31.32		**<0.001**	
	Air temperature	0.00	0.00		0.985		-0.05	0.08		0.787	
	Air humidity	0.24	0.62		0.451		0.20	0.57		0.468	
Whole day activity (05:00 to 19:00)**				0, 14		0.00			0, 14		0.00
	Air temperature	-0.03	0.01		0.916		-0.08	0.09		0.770	
	Air humidity	-0.37	2.06		0.175		-0.39	2.32		0.151	
	Light intensity	0.06	0.05		0.825		0.04	0.02		0.886	

* Marginally significant statistic value.

** Model with no effects of environmental factors (see explanation in the statistical analysis section).

Table 2. Descriptive statistics of temporal and spectral traits of the advertisement call of the diurnal stream frog *Crossodactylus schmidti* from southern Brazil. N/C = notes per call; CD = call duration; N/s = notes per second; NDF = note dominant frequency; NFM = note frequency modulation; FBH1 = frequency bandwidth of harmonic 1; FBH2 = frequency bandwidth of harmonic 2; FBH3 = frequency bandwidth of harmonic 3; SPL = peak sound pressure level.

Trait	Mean	SD	Minimun	Maximum	Sample size (10 males)
NC	31	7	13	44	25 calls
CD (s)	4.17	0.73	2.28	5.63	25 calls
N/s	7	1	6	10	25 calls
NDF (Hz)	3244	501	2186	5172	357 notes
NFM (Hz)	807	400	190	1721	25 calls
FBH1 (Hz)	2592	311	2186	3109	61 notes (17%)
FBH2 (Hz)	3312	366	2245	4315	269 notes (75%)
FBH3 (Hz)	4034	366	3706	5172	27 notes (8%)
Note SPL (dB)*	73	2	63	77	269 notes
Noise SPL (dB)*	68	4	58	76	269 note intervals

*At the frequency range of the second harmonic, where NDF is more often found.

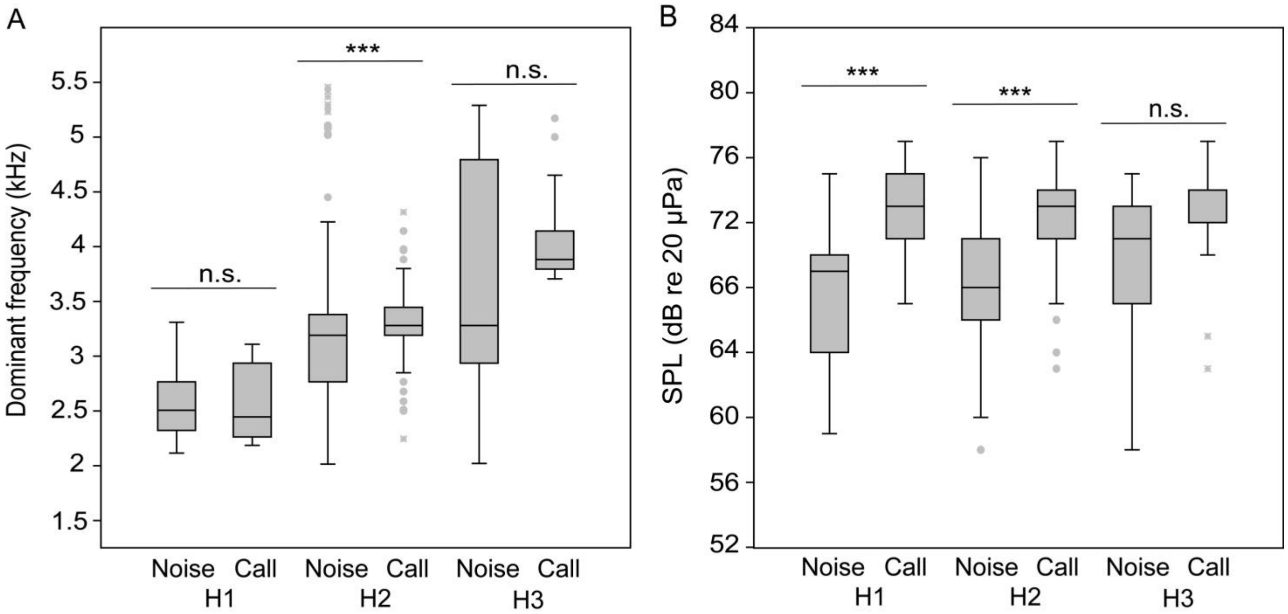

Figure 3. Variation in A) the dominant frequency and B) peak sound pressure level (SPL) of the acoustic signal and the ambient noise filtered at note dominant frequency in different call harmonics (H1, H2 and H3). Boxes refer to the 25-75 percent quartiles with the median as horizontal lines inside, minimum and maximum values shown as whiskers and extreme values (not outilers) as circles. Results of the Mann-Whitney pairwise post-hoc comparisons – “Call vs. Noise, Dominant frequency”: H1 (*U*= 1776, *P*= 0.10); H2 (*U*= 2800, *P*<0.0001); H3 (*U*= 252, *P*= 0.803) – “Call vs. Noise, SPL”: H1 (*U*= 528, *P*<0.0001); H2 (*U*= 1345, *P*<0.0001); H3 (*U*= 226, *P*= 0.239). Asterisks indicates a significant difference (*P*<0.0001; Bonferroni corrected probability values) and n.s. a non significant difference.

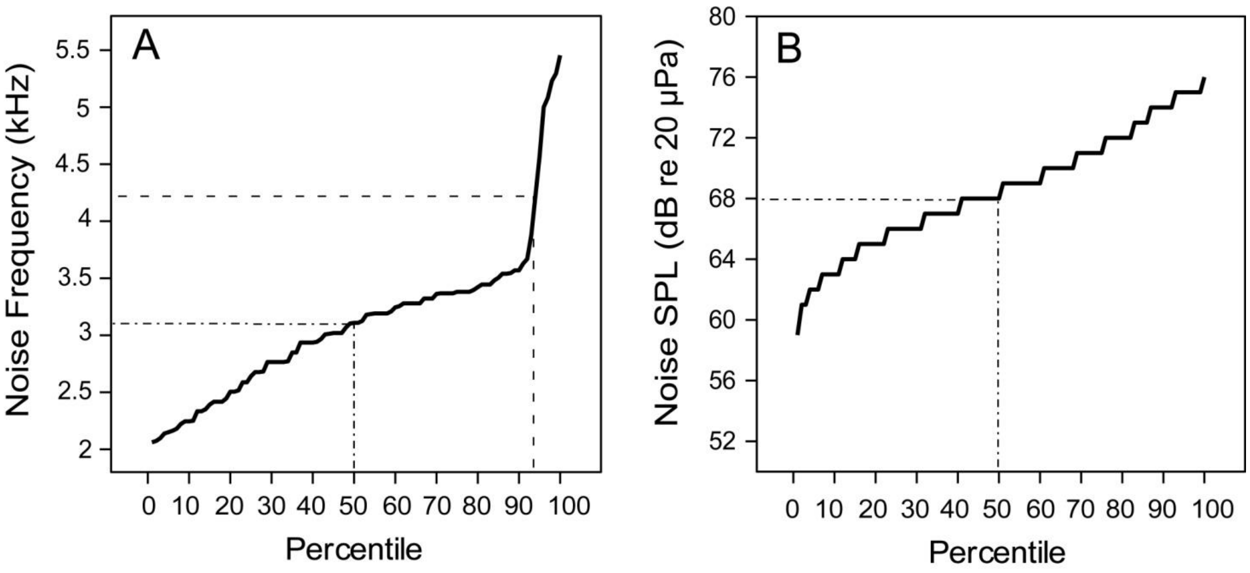

Figure 4. Percentiles of variation in A) dominant frequency and B) SPL of ambient noise filtered at call dominant frequency. Dashed-dotted lines represent the arbitrary interval of 50% of variance in noise for visual reference, whereas the dashed line in A represents the interval where noise frequencies overlap within the range of the second harmonic, where NDF is more often located.

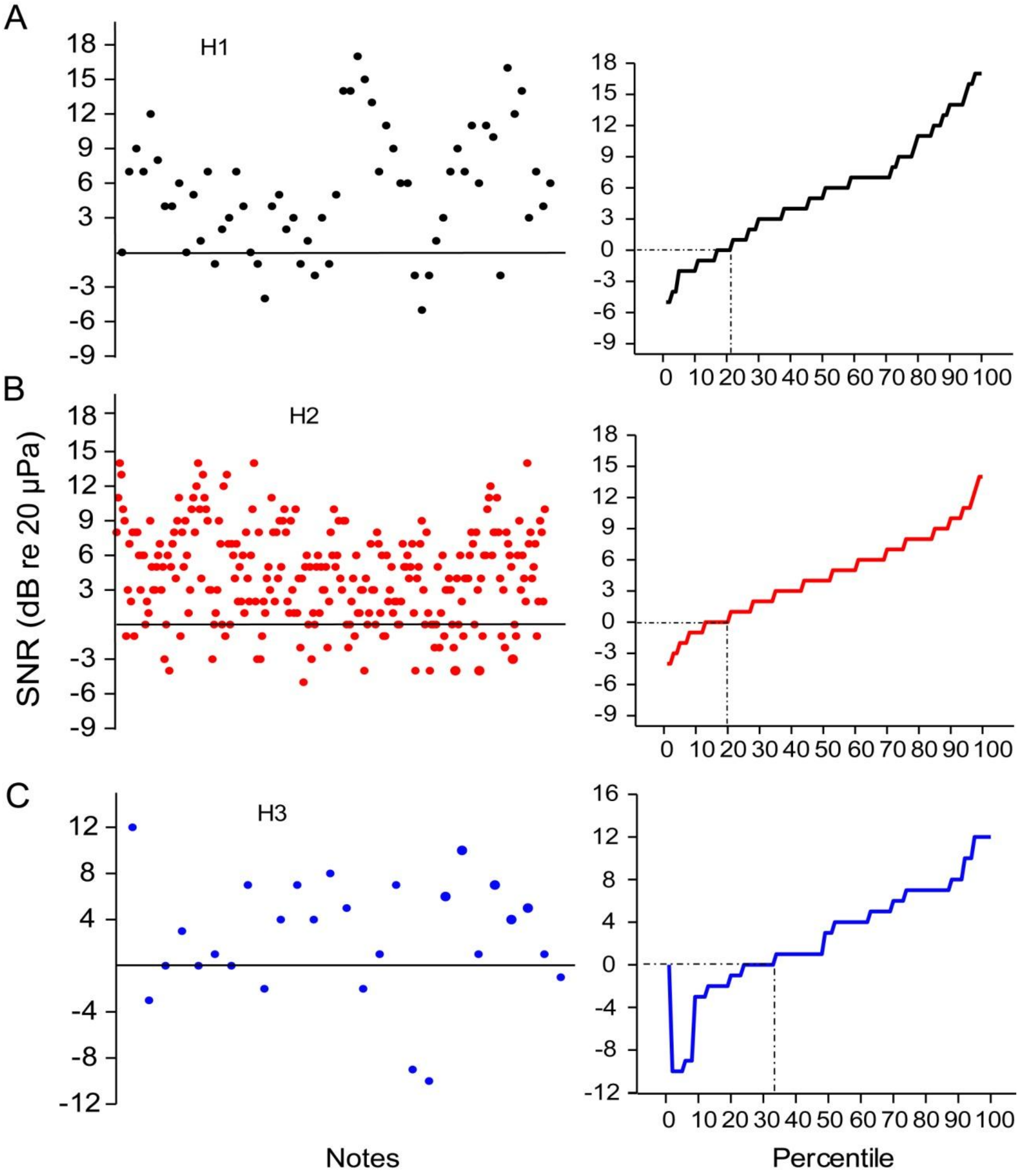

Figure 5. Signal-to-noise relations between notes of the advertisement call of *Crossodactylus schmidt* and the ambient noise of streams in southern Brazil. A) Difference (in dB) between note and noise SPL, with noise filtered at the first harmonic (H1, black), and percentiles of variation in the signal-to-noise ratio (SNR) for H1; B) Difference between note SPL and noise SPL, with noise filtered at the second harmonic (H2, red), and percentiles of variation in the SNR for H2; C) Difference between note SPL and noise SPL, with noise filtered at the third harmonic (H3, blue), and percentiles of variation in the SNR for H3. Overall, 22% of notes had a SNR ≤ 0 (n = 77) and 78% had a SNR > 0 (n = 280). Dashed boxes in the percentile graphs refer to the percentile of notes with SNR ≤ 0, for each harmonic.

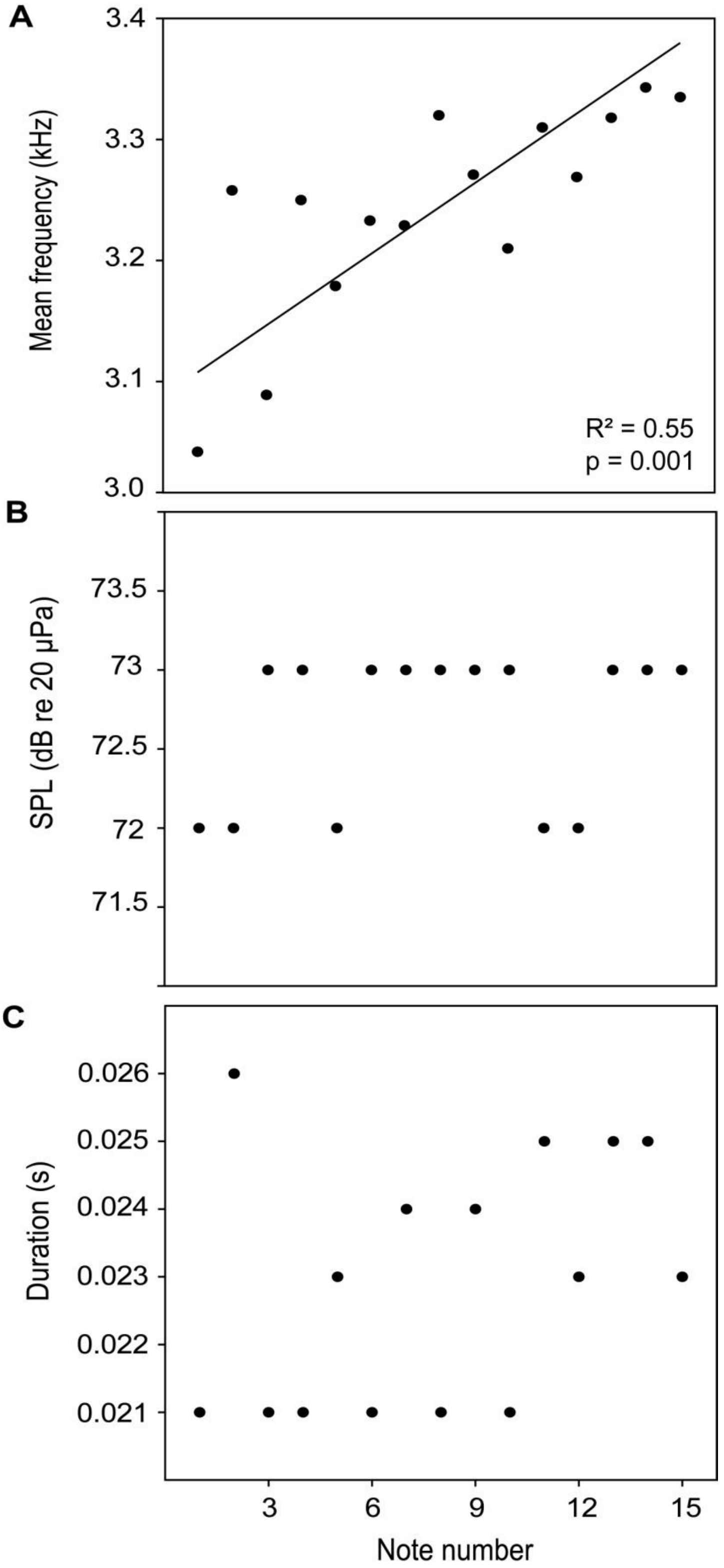

Figure 6. Variation in call traits along 15 notes representing the beginning, middle and the end of the of the advertisement call of *Crossodactylus schmidti.* A) Mean note frequency; B) mean note SPL; C) mean note duration.

4. Discussion

Our study revealed three major results regarding the acoustic communication of *Crossodactylus schmidti*: *1) C. schmidti* had a continuous pattern of calling activity at the study site, with active males occurring year-round despite the typical climatic seasonality of southern Brazil; *2)* environmental factors had variable effects in the monthly and daily patterns of calling activity; and 3) some traits of the spectral structure of the acoustic signal showed a degree of plasticity related to ambient noise variation, whilst other characteristics of the communication system of *C. schmditi* are regarded as long-term, evolutionary adaptations.

4.1. Temporal patterns of calling activity

A common temporal pattern of reproduction observed in southernmost communities of nocturnal frogs in the Neotropics is a concentrated calling activity in austral spring and summer, correlated with longest photoperiods and highest temperatures (Both et al. 2008; Canavero et al. 2008; Canavero and Arim 2009). This pattern was also observed for most of individual species within a community (Both et al. 2008) and for the habitat specialist frog *Limnomedusa macroglossa* (Kaefer et al. 2009). These factors are known to act as regulators of biological, hormonal rhythms related with reproduction, i.e., calling activity (Hatano et al. 2002; Almeida-Gomes et al. 2007b) and gametogenesis (Wells 2007). Contrary to the above-mentioned studies, however, we found that the calling activity of *C. schmidti* did not present a significant mean direction over a one-year period; instead, males were active throughout the year and the photoperiod did not affect the number of calling males and calls, nor the extension of calling activity. In support of this pattern, another study conducted with *C. schmidti* in the same area revealed continuous gonadal activity in both sexes and free-swimming tadpoles occurring year-round (Caldart, unpublished data). Our results also differ from those found for *Hylodes phyllodes* at a tropical area of southeastern Brazil, where males did not call in the months of the dry season with shortest photoperiod for two consecutive years (Hatano et al. 2002). In the same area, however, males of *Crossodactylus gaudichaudii* were engaged in calling activity year-round (Almeida-Gomes et al. 2007b), yet both studies indicated photoperiod as a strong regulator of calling activity extension.

Our models showed that the monthly calling activity of *C. schmidti* was positively affected by accumulated rainfall and air temperature. The former factor significantly affected the number of calling males, also marginally affected by air temperature, whereas both factors had significant effects on the number of calls year-round. The effect of accumulated rainfall on male activity may be linked to the reproductive mode of *Crossodactylus* species (mode #3 *sensu* Haddad and Prado, 2005), whose males defend territories and excavate pebbles under rocks to access sub aquatic chambers where the eggs are laid and tadpoles develop (Weygoldt and Silva 1992; Wells 2007). We observed considerable variation in water flow at our study stream due to variation in rainfall amounts along the year. Strong rainfall episodes may impair acoustic communication due to increased ambient noise (Brumm and Slabbekoorn 2005), and we observed that males completely ceased calling activity in such cases. Besides, hydrological disturbance may change the spatial dynamics of streams from few to several days (Lake 2000), by potentially displacing rocks used as calling sites and the bottom sediment which males have to dig to get access to oviposition sites. We believe this is the reason why the longer rainfall variable (15 days accumulated rainfall) affected males' activity rather than the shorter one. Moreover, the few evidence on the development of larvae of *Crossodactylus* indicates that early-stage tadpoles stay restricted to the chambers, as only large free-swimming tadpoles were observed and collected in captivity (Weygoldt and Silva 1992) and in nature (Caldart, unpublished data). Air temperature, in turn, is well known to influence nearly all biological processes, including anuran calling performance (Wells 2007). Hence, the effect of air temperature on the number of calls – and the marginally significant effect of this variable on the number of calling males year-round – are likely related with the high metabolic rate of calling activity, given that anurans depend on external temperature to maintain body activity (Wells and Taigen 1986; Wells 2001). Air temperature also affected the monthly activity of *Hylodes phyllodes* in southeastern Brazil (Hatano et al. 2002).

The daily calling activity of *C. schmidti* was positively affected by air temperature at the early morning hours and by light intensity at the rest of the daytime. The peak of calling activity in the early morning is in accordance with the results found for ecologically similar species of frogs. From the Atlantic Forest of the Neotropics to forests of India and Borneo, some diurnal stream frogs have a peak of activity in the early morning, starting even before sunrise (Hatano et al. 2002; Almeida-gomes et al. 2007; Grafe and Wanger 2007; Preininger et al. 2013b). Nevertheless, the early morning peak of activity of *C. schmidti* was much more pronounced and occurred in all months. A possible explanation is that reduced atmospheric turbulence at dawn favors acoustic signal transmission over long ranges by minimizing signal degradation, though studies on this topic with birds have revealed that signaling at dawn is more advantageous in open habitats than in forests (Wiley and Richards 1978; Brown and

Handford 2003). Studies on transmission and degradation of acoustic signals of diurnal stream frogs are still needed to further investigate fine-scale temporal variability in calls emission. Besides, the early morning models had the lowest explained variation amongst our models; therefore we should consider that other factors may affect calling activity at the early morning, e.g., the circadian rhythm. Regarding the remaining daytime activity of *C. schmidti*, as we have predicted, an increase in light intensity at calling sites increased both the number of calling males and number calls, possibly because it favors the discrimination of the sender and its visual signals to intended receivers. This is likely the main reason why light intensity affects the calling activity of diurnal stream frogs known to widely use acoustic (Hatano et al. 2002; Almeida-gomes et al. 2007; Caldart et al. 2014), visual and audiovisual signals (Caldart et al. 2014). Males of the Bornean rock frog *Staurois guttatus*, for instance, communicate predominantly through acoustic signals during the early morning, shifting to a prevalent use of visual signals as ambient light increases in calling sites (Grafe and Wanger 2007).

The above-mentioned effects of environmental factors on calling activity may have implications in the mating system of *C. schmidti* which are worth to be briefly discussed in order to stimulate further studies. Given that the selection of oviposition sites in nature occurs in a heterogeneous temporal and spatial context (Silva and Giaretta 2008) and assuming that the frequent, long agonistic interactions between males (Caldart et al. 2014) are contests for adequate calling and oviposition sites, one could argue that selection of adequate calling and oviposition sites – whose availability is affected by hydrological dynamics due to rainfall variation – and male calling performance, in turn affected by air temperature, could constitute potential traits used by females for mate selection. Therefore, we propose that the mating system of *C. schmidti* fits into the resource defense polygyny type (*sensu* Emlen and Oring 1977) and rely primarily on female choice based on the quality of resources defended by males, i.e. oviposition sites (*sensu* Silva and Giaretta 2008), and secondarily on male calling performance, i.e. selection based on individual characteristics of call traits.

4.2. *Call structure and signal-to-noise relations*

The advertisement call of *C. schmidti* greatly varied in spectral traits, with most noticeable variation occurring in note dominant frequency (NDF). The location of NDF varied among harmonics, therefore resulting in a high note frequency modulation (NFM). Furthermore, NDF increased on average 300 Hz along call notes. An average increase of 300

Hz in note frequency along call was also reported for another diurnal stream frog, *Staurois parvus*, though males also increased note amplitude and note duration throughout the call (Grafe et al. 2012). Animals are known to show plasticity in signal emission in order to cope with background noise (Brumm and Slabbekoorn 2005), and for the case of *C. schmidti*, the increase in NDF along call must facilitate signal detection to nearby and distant receivers, given that *1)* calling males presumably cannot accurately evaluate the ambient noise of sites farther away from their position, and that *2)* frequency modulation is preferable than amplitude modulation of an air-borne acoustic signal, because sound frequency is less affected by habitat reverberation and turbulence than is sound amplitude (Wiley and Richards 1978; Endler 1993). Therefore, we interpret the variation in spectral traits – the note frequency modulation with variable location of NDF among harmonics, and the increase in NDF along call notes – as short-term behavioral shifts related with temporary, subtle changes in ambient noise (*sensu* Brumm and Slabbekoorn 2005). On the other hand, the lack of correlation between temporal call traits and habitat noise reinforce the idea that variation in temporal call traits must be more dependent on social interactions than on ambient noise (Caldart et al. 2014).

The signal-to-noise investigation showed that *1)* with noise filtered at the first harmonic, call and noise frequency did not differ, but call SPL was significantly higher than noise SPL; *2)* with noise filtered at the second harmonic, both frequency and SPL were significantly higher for calls than for noise; and *3)* with noise filtered at the third harmonic, neither frequency nor SPL differed between call and noise. Thus, the first and the second harmonics seems to provide free-of-noise channels for communication, given that when dominant frequency of call and noise overlapped, the amplitude did not (as the case of the first harmonic). This pattern could lead one to assume that the harmonic call of *C. schmidti* is not precluded by ambient noise, but we argue that this notion may be simplistic as it does not hold true if we consider the signal-to-noise ratio along call notes. As *C. schmidti* emits multi-note calls with variable location of NDF among harmonics, we also asked if the signal-to-noise ratio (SNR) varied along call notes and at different harmonics. Remarkably, we found that all males emitted calls composed by a few to some notes with negative SNR at all harmonics (SNR ≤ 0), which means that part of their call is indeed masked by habitat noise, regardless of the harmonic containing the NDF. In accordance with recent field and experimental studies (Cunnington and Fahrig 2010; Love and Bee 2010), this result presents new *in situ* evidence that anurans are not able to increase call amplitude as a function of ambient noise, i.e. the Lombard Effect (Brumm and Zollinger 2011). As discussed above, instead of amplitude modulation of notes we observed an increase in note frequency along call, reinforcing the idea that noise-dependent regulation in anuran calls must be more common on traits other than signal amplitude.

The dominant frequency of anuran calls have a phylogenetic effect because it is inversely related with body size (Gingras et al. 2013), and thus the low-frequency noise of lotic habitats may act as a filter selecting for small-sized species breeding alongside streams (Vargas-Salinas and Amézquita 2013). For species with harmonic calls which inhabits streams, the dominant frequency located at higher harmonics have been regarded as an acoustic adaptation to the masking effect of the low-frequency ambient noise, but most studies did not investigate signal-to-noise relations, particularly for Hylodidae species (e.g., Haddad and Giaretta 1999; Pimenta et al. 2008). In one direction our results are consistent with the argument of convergent evolution of high-frequency calls due to the historical pressure of habitat noise (Grafe et al. 2012; Preininger et al. 2013a), though on the other hand they suggest that higher harmonics do not always present higher SNR, contrary to what have been discussed for Hylodidae species (Haddad and Giaretta 1999; Lingnau and Bastos 2007; Pimenta et al. 2008; Caldart et al. 2011). In the light of our new evidence, it seems reasonable that the NDF in calls of *C. schmidti* is more often located at the second harmonic, rather than at the third, because at this range sound propagation is favorable as intermediate frequencies suffer much less attenuation than frequencies below 1–2 kHz and above 4 kHz (Wiley and Richards 1978).

Besides having a harmonic structure, the calls of *C. schmidti* are composed of several notes (Caldart et al. 2011). Multi-note calls have evolved because redundant signals provide a greater contrast with background noise than do signals composed by one or a few notes (Endler 1992; Wells 2007). Moreover, short-term behavioral shifts may be used to increase call redundancy and increase signal detection in noisy environments, e.g. increased note duration, call duration or call rate (Brumm and Slabbekoorn 2005), but our results did not indicate the use of temporal adjustments in the calls of *C. schmidti* related with ambient noise variation. Given that all males of *C. schmidti* emitted calls with negative SNR, we suggest that the multi-note call of *C. schmidti* may have evolved because repetitive notes increase signal redundancy, diminishing the negative effect of having some notes precluded by habitat noise. Likewise, the use of visual signals alone or within a audiovisual display (Caldart et al. 2014) may also have been favored in the communication system of *C. schmidti* due to the masking effect of the ambient noise on its acoustic signal transmission.

4.3. *Concluding remarks*

Our results indicate a continuous calling activity of *C. schmidti* throughout the year in southern Brazil, with different effects of environmental factors on the monthly and daily patterns. Accumulated rainfall and air temperature are the main factors affecting monthly calling activity, whereas daily activity is mainly affected by air temperature and light intensity. We suggest that the monthly pattern of activity and its relation with accumulated rainfall are tight linked with the species reproductive mode, which imposes a selective pressure on males' selection of calling and oviposition sites. Male's abilities to select and maintain territories could be explored for mate selection by females, which may also evaluate male calling performance – affected by air temperature – during mate choice. On the other hand, the early morning pattern of calling activity may be related with efficacy of signal transmission, i.e. acoustic signal transmission is favored by more stable atmospheric conditions in the early morning, though factors such as circadian rhythm must also play a role. Finally, regarding the remaining daytime activity and its relation with light intensity, the transmission of visual signals alone or within a audiovisual display is likely favored by light intensity reaching male calling sites along the day, as also reported for close-related species (Hatano et al. 2002; Almeida-gomes et al. 2007).

With respect to the signal-to-noise relations, although the significant difference between the SPL of the acoustic signal and the ambient noise filtered at NDF, some notes of the calls of all males of *C. schmidti* had negative SNR. In this sense, characteristic such as *1)* the multi-note call, *2)* the call harmonic structure with NDF usually in the second harmonic, and *3)* the use of visual and audiovisual signals, must have evolved due to the pressure of constant ambient noise, and are therefore interpreted as long-term evolutionary adaptations (*sensu* Brumm and Slabbekoorn 2005). Differently, variation in signal spectral structure such as the *1)* the note frequency modulation with variable location of NDF among harmonics, and *2)* the increase in NDF along call notes, are interpreted as short-term behavioral shifts dependent on temporary, subtle changes in ambient noise (*sensu* Brumm and Slabbekoorn 2005).

In conclusion, our study provides a first attempt in the understanding of signal-to-noise relations for a Neotropical diurnal stream frog, providing valuable insights into noise-dependent adjustments in call traits and signal evolution of anurans inhabiting streams. Given the convergent evolution of high frequency multi-note calls and visual displays in diurnal stream frogs (Haddad and Giaretta 1999; Grafe et al. 2012; Preininger et al. 2013a), additional studies with species of *Hylodes* and *Crossodactylus* should investigate signal-to-noise relations to uncouple noise-dependent evolutionary adaptations and behavioral shifts in calls traits. Future studies addressing fine-scale temporal patterns of calling activity and signal-to-noise relations in diurnal stream frogs, will hopefully increase our knowledge on evolutionary adaptations and short-term strategies of communication in acoustically complex environments.

Acknowledgments

We are grateful to Doris Preininger for kindly discussing the bioacoustic procedure of signal-to-noise analysis, to Camila Both, Cynthia Prado, Selvino Neckel de Oliveira and Sandro Santos for valuable suggestions that improved this study, and to the staff of the Laboratório de Herpetologia – UFSM for their assistance in field activities. V.M.C. and S.I. thank CAPES for the doctoral fellowships and S.Z.C. is grateful to CNPq for the award of a research fellowship (No. 304929/2012-3). Activities in Turvo State Park were performed in accordance with the access licenses (Nos 133/2010 and 23/2011) issued by SEMA-RS.

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Supplementary material

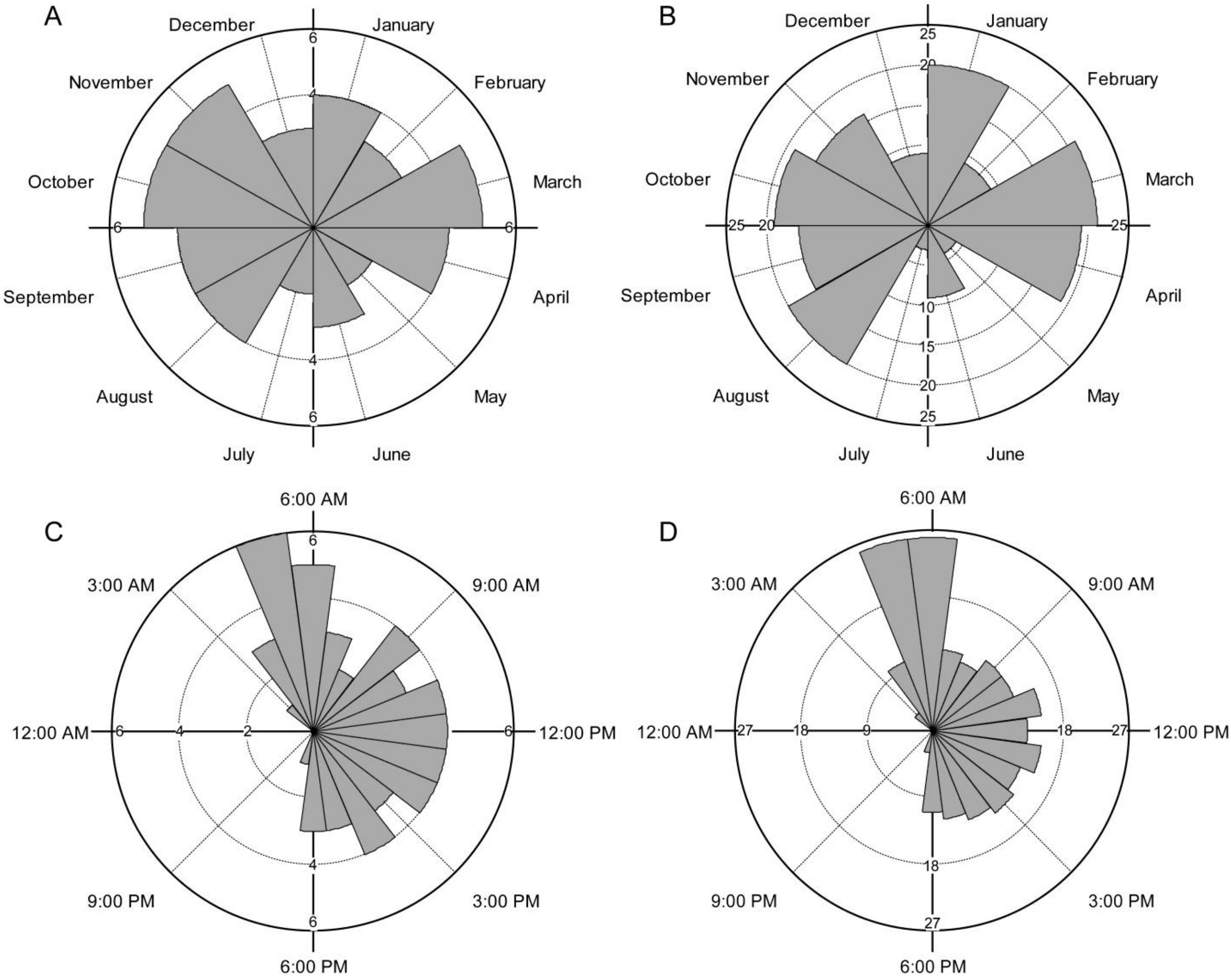

Figure S1. Circular diagrams of the calling activity of *Crossodactylus schmidti* in southern Brazil. A) mean number of calling males registered at monthly intervals; B) mean number of calls registered at monthly intervals; C) mean number of calling males registered at hourly intervals; D) mean number of calls registered at hourly intervals.

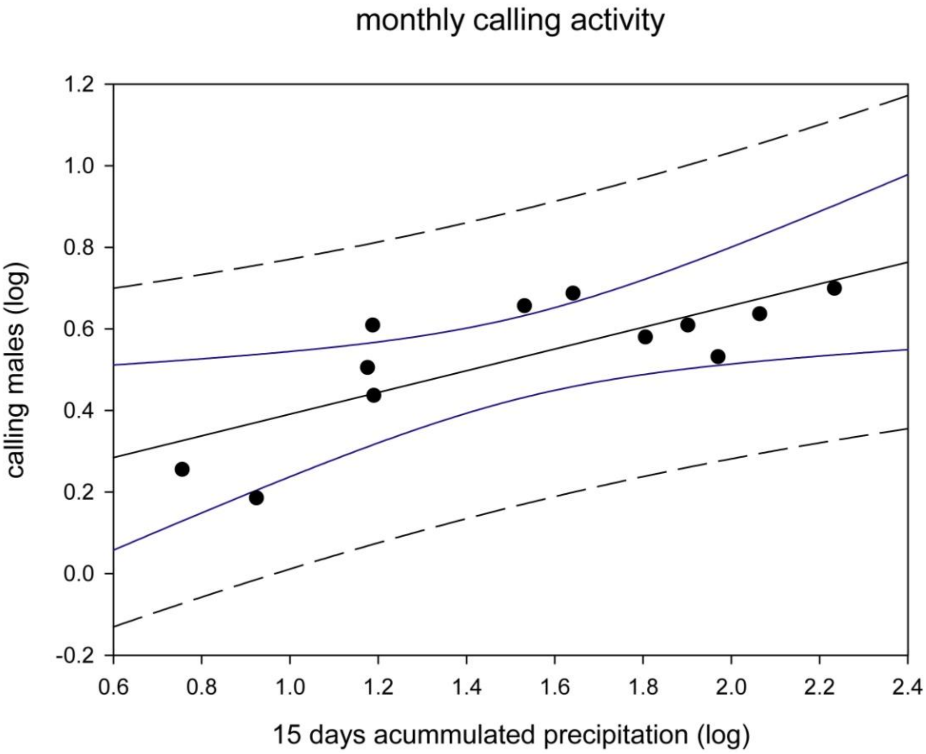

Figure S2. Relationship between monthly number of calling males of *C. schmidti* and 15 days accumulated rainfall in southern Brazil. Dots represent mean values for each monthly interval, blue lines represent 99% confidence interval and dashed lines refer to the prediction intervals.

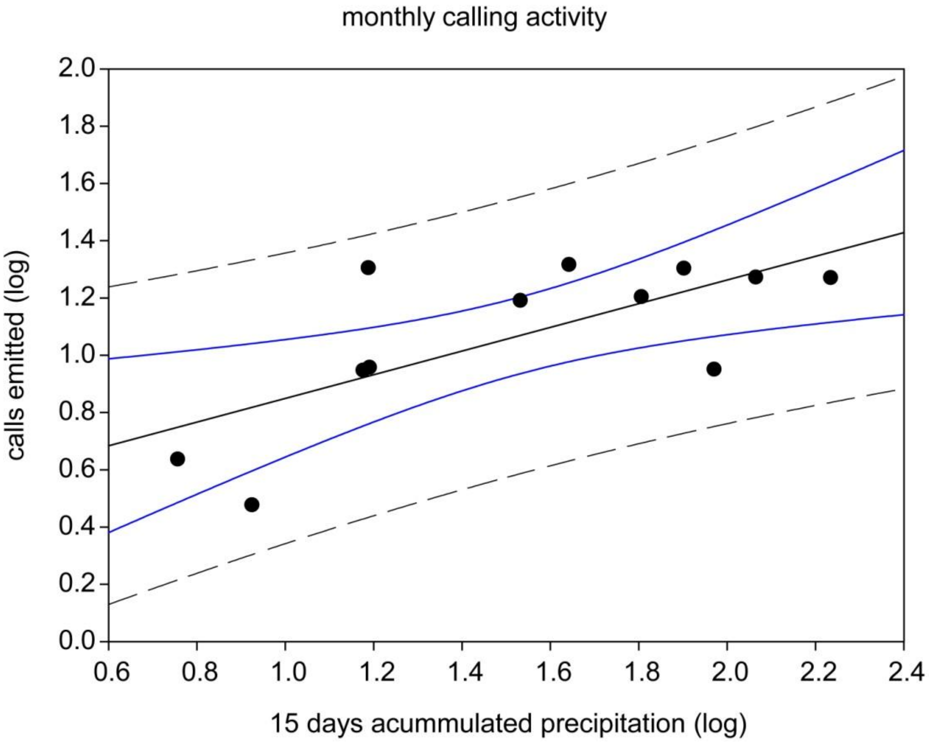

Figure S3. Relationship between monthly number of calls of *C. schmidti* and 15 days accumulated rainfall in southern Brazil. Dots represent mean values for each monthly interval, blue lines represent 99% confidence interval and dashed lines refer to the prediction intervals.

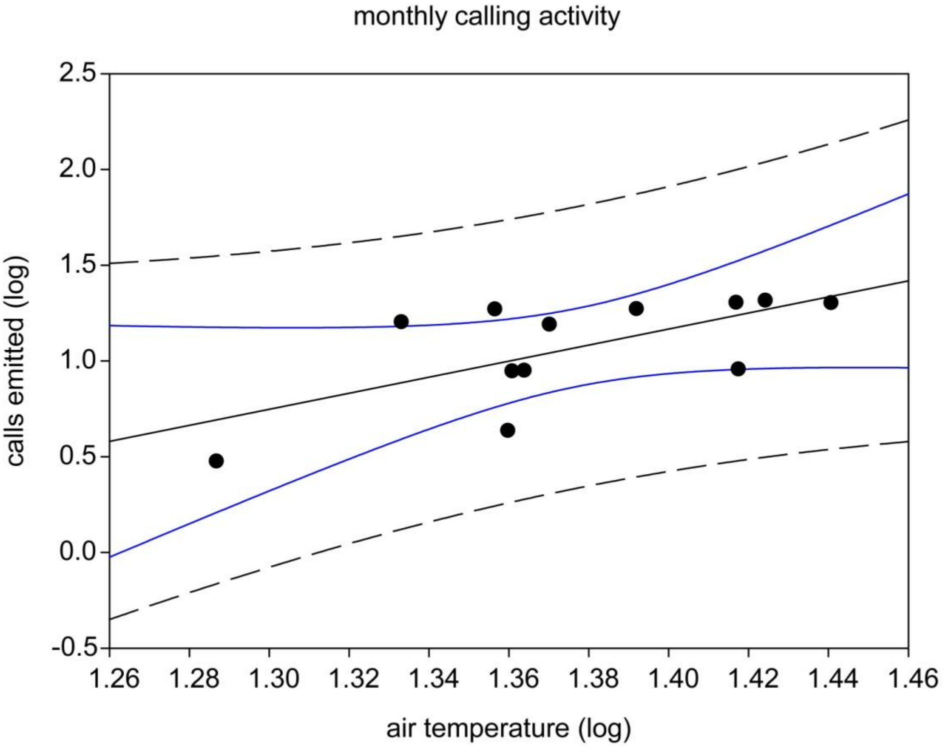

Figure S4. Relationship between monthly number of calls of *C. schmidti* and air temperature in southern Brazil. Dots represent mean values for each monthly interval, blue lines represent 99% confidence interval and dashed lines refer to the prediction intervals.

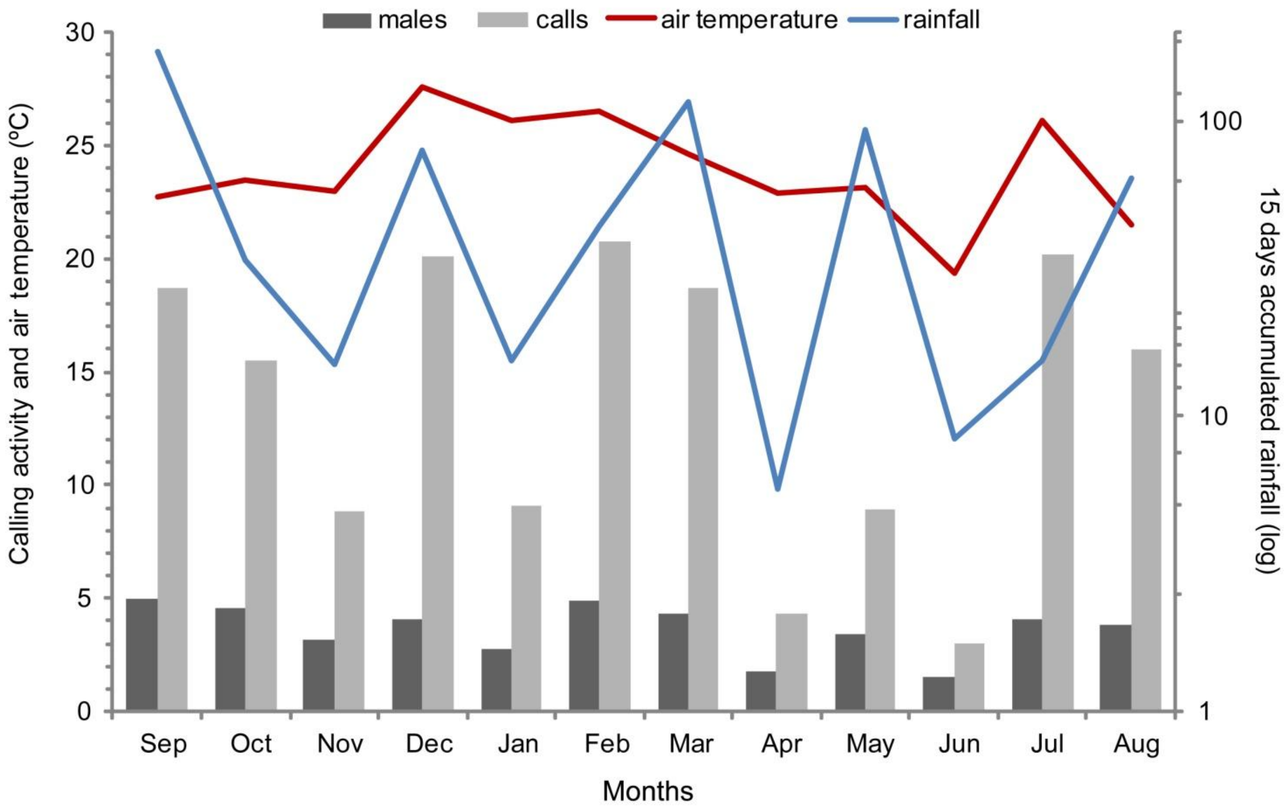

Figure S5. Variation in the monthly mean calling activity of *C. schmidti* and environmental factors in southern Brazil.

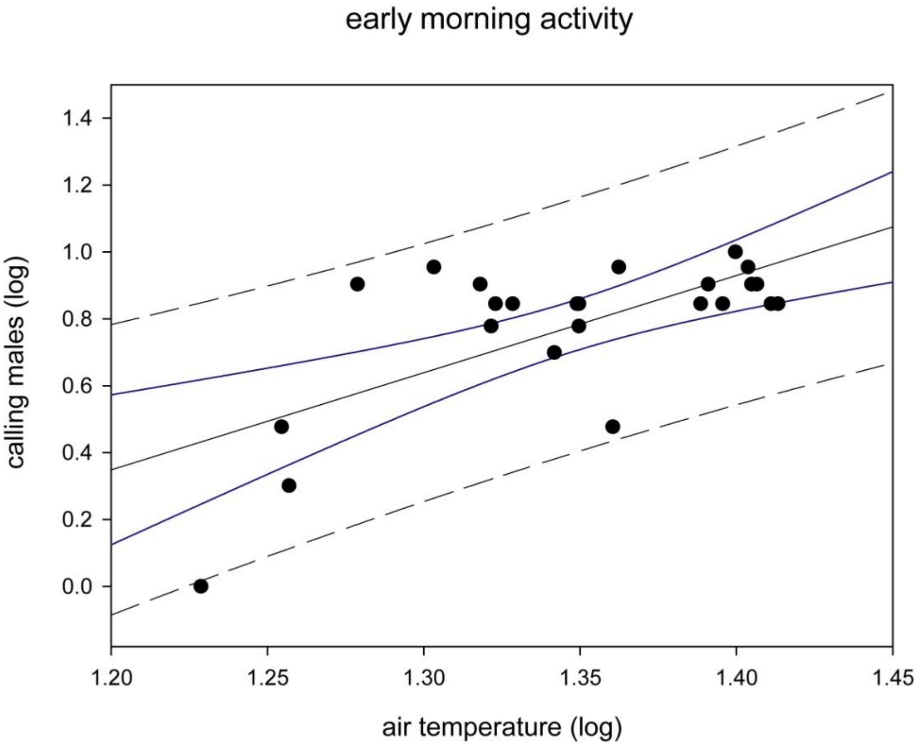

Figure S6. Relationship between number of calling males of *C. schmidti* and air temperature for the early morning hours in southern Brazil. Dots represent mean values for each hourly interval between 05:00 and 06:00, blue lines represent 99% confidence interval and dashed lines refer to the prediction intervals.

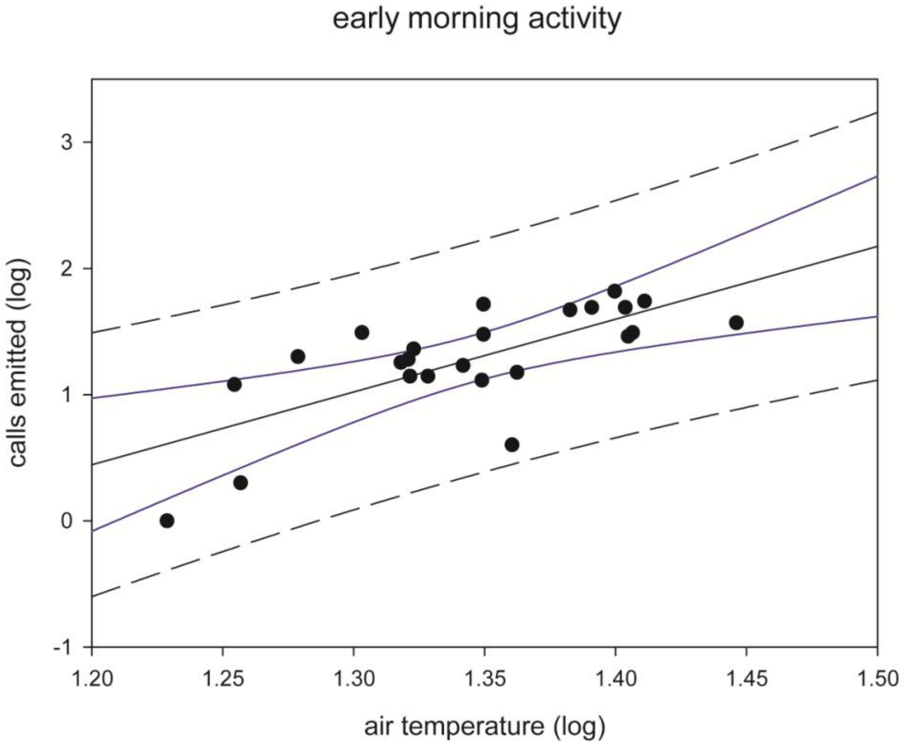

Figure S7. Relationship between number of calls of *C. schmidti* and air temperature for the early morning hours in southern Brazil. Dots represent mean values for each hourly interval between 05:00 and 06:00, blue lines represent 99% confidence interval and dashed lines refer to the prediction intervals.

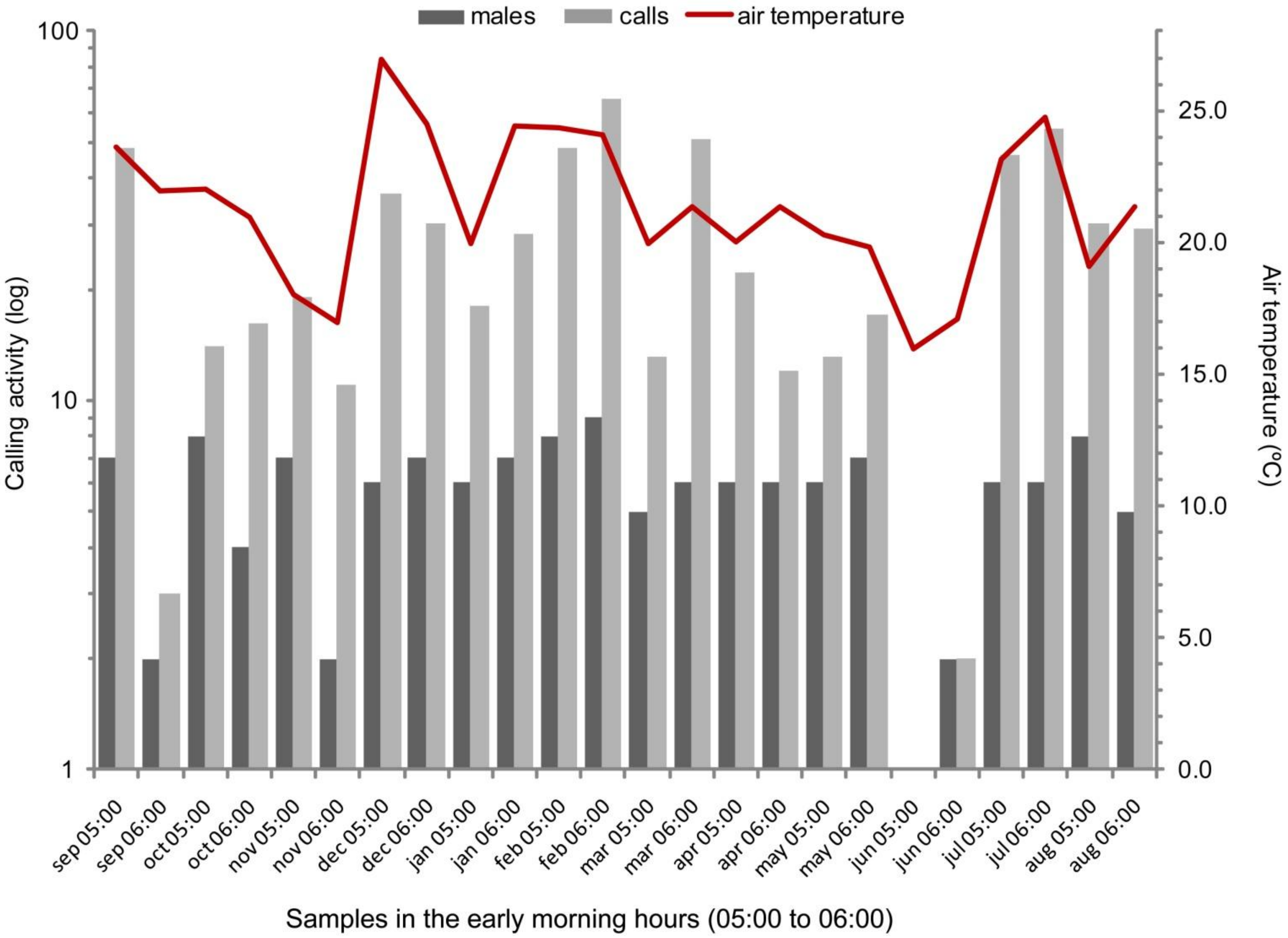

Figure S8. Variation in the early morning calling activity of *C. schmidti* and environmental factors in southern Brazil.

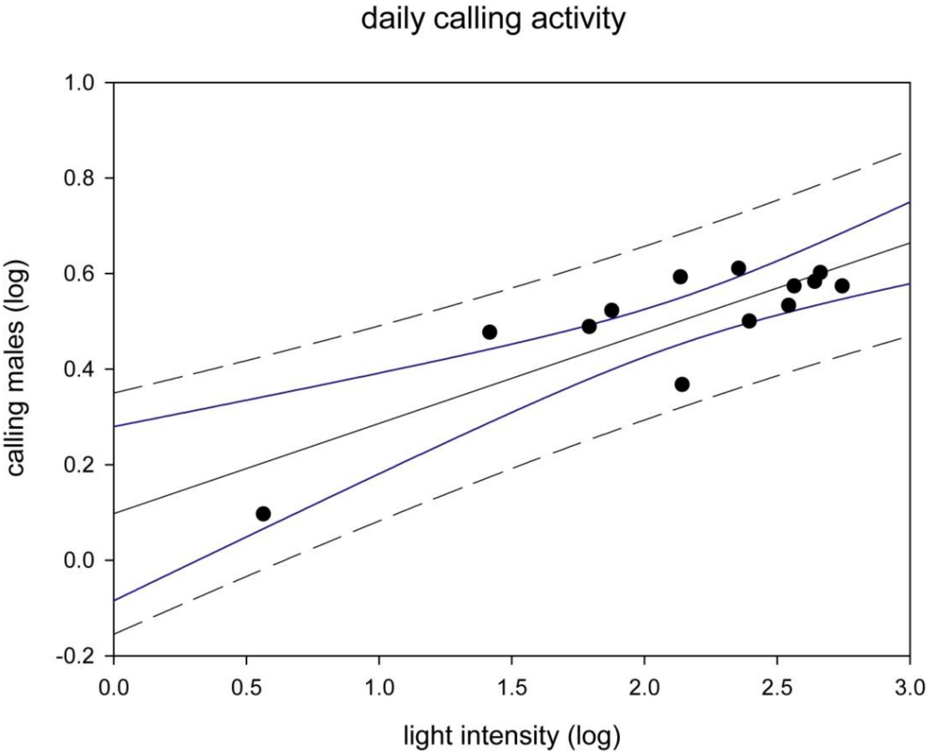

Figure S9. Relationship between daily number of calling males of *C. schmidti* and light intensity in southern Brazil. Dots represent mean values for each hourly interval between 07:00 and 19:00, blue lines represent 99% confidence interval and dashed lines refer to the prediction intervals.

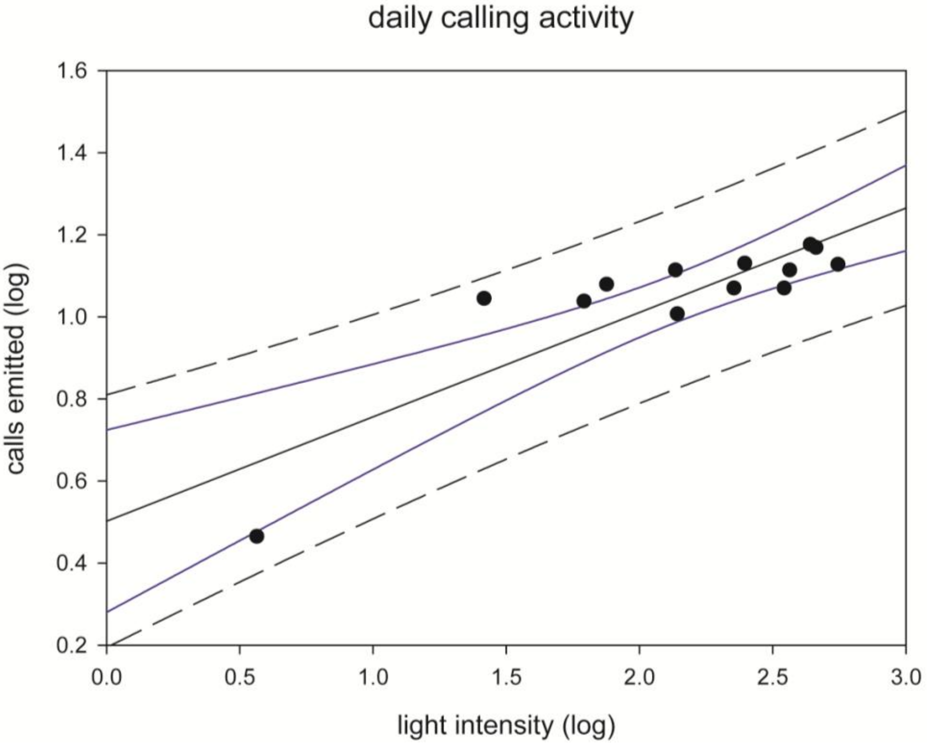

Figure S10. Relationship between daily number of calls of *C. schmidti* and light intensity in southern Brazil. Dots represent mean values for each hourly interval between 07:00 and 19:00, blue lines represent 99% confidence interval and dashed lines refer to the prediction intervals.

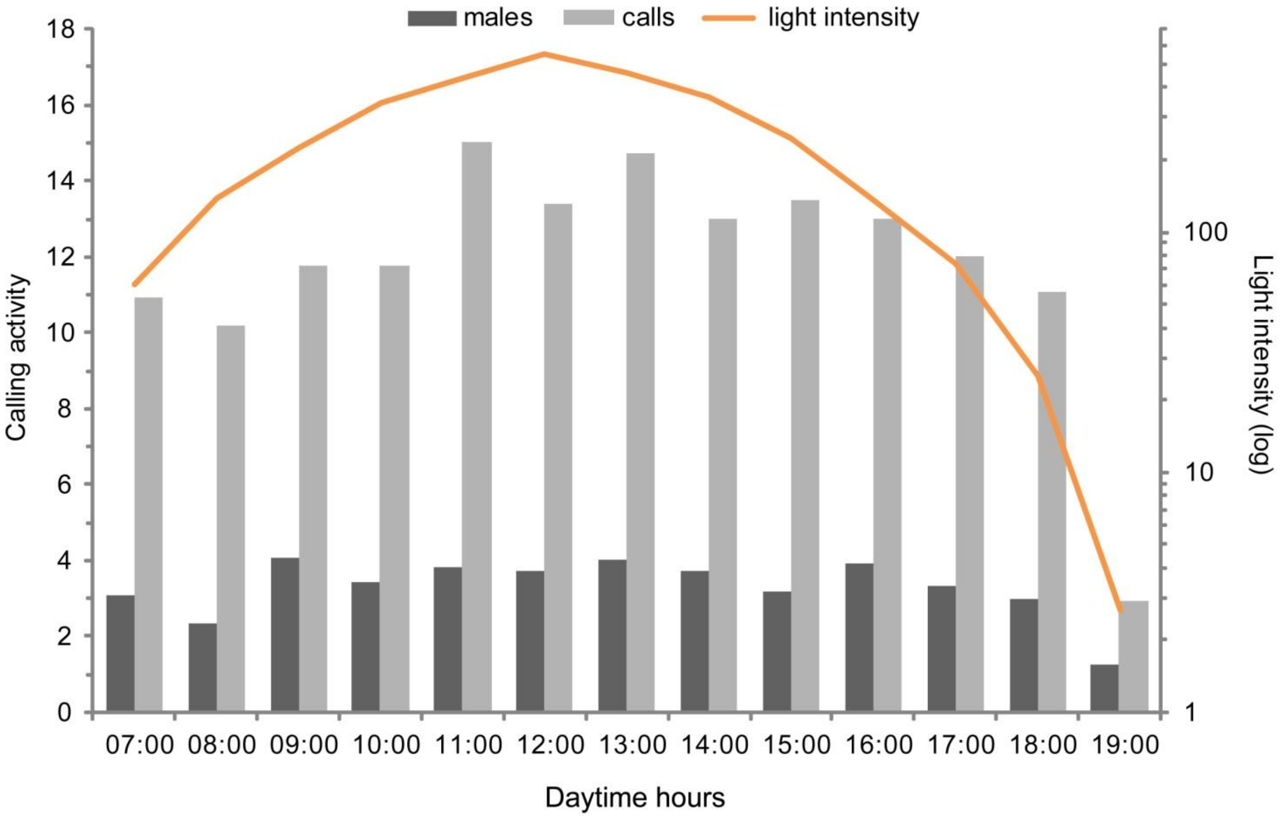

Figure S11. Variation in the daytime calling activity of *C. schmidti* and environmental factors in southern Brazil.

CAPÍTULO IV

Continuous reproductive cycle in males and females of *Crossodactylus schmidti* (Anura, Hylodidae) in the subtropical climate of southern Brazil

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Continuous reproductive cycle in males and females of *Crossodactylus schmidti* (Anura, Hylodidae) in the subtropical climate of southern Brazil

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Abstract. The temporal strategies of reproduction are influenced by a combination of intrinsic factors and environmental conditions. Climatic factors often explain the calling activity of anurans, but little is known about basic life history aspects linked with reproduction for diurnal stream breeders, such as seasonal variations in gametogenic cycles, size-fecundity relationships and sexual dimorphism. We investigated the reproductive biology of the diurnal stream frog *Crossodactylus schmidti* through histological analysis of gonads from specimens collected between 2009 and 2012 in southern Brazil. Mature individuals of both sexes occurred in all months, though the proportion of mature individuals along the year varied more for females than for males. Free spermatozoa, sperm bundles and germ cell cysts in several stages were found in testes of all except two males (n = 44; 96%), whereas ovaries of most of the females presented all follicle stages (n = 65; 71%). There was no significant seasonal variation neither for males nor females in snout-vent length (SVL), body mass (BM) or in the gonadosomatic index. Though BM had a significant positive effect on gonad mass for both sexes, the effect was stronger for females. Mature females were significantly larger in SVL than males. After controlling the effect of SVL on other morphological traits, we found sexual dimorphism only in mouth width (MW), with females having larger MW. Besides, we collected free-swimming tadpoles and registered males in calling activity year-round. Our results indicate that *C. schmidti* has a continuous pattern of reproduction in subtropical Brazil, despite of the local climatic seasonality, with gametogenesis, calling activity and free-swimming tadpoles occurring year-round. The histological evidence support the argument of a strong intrasexual competition, since mature individuals of both sexes are available in all months, with variable proportion of mature females along the year, which explain why males continuously compete for territories. Though males and females may reach sexual maturity at small sizes, larger individuals must be in advantage for reproduction, as larger males should be benefited at establishing and defending calling/oviposition sites related with the species reproductive mode and mating system, and larger females must benefit more than smaller females by having a potentially larger spawn.

Keywords: Reproduction, spermatogenesis, oogenesis, reproductive investment, continuous reproduction, sexual dimorphism.

1. Introduction

The study of the evolution of life histories is a search for patterns and explanations for many key characteristics of an organism's life cycle, such as the patterns of growth, differentiation, storage and use of resources and reproduction. One can investigate life history among specific life history traits (e.g., hatchling size in different organisms), between different life history traits (e.g., relation between sexual maturity and longevity) and between life history traits and habitat characteristics (Roff 1992; Stearns 1992). An successful approach in the understanding of the evolution of life history traits relies in the concept of "optimization", which establishes that the combination of the observed components of the life history of a given organism is assumed as that which provide higher adaptive values to that organism (Stearns 2000), though individual fitness may also be "frequency-dependent", when selection for a given trait vary with population density (Sinervo et al. 2000). As directly related to individual fitness, reproduction is a key aspect of life history of organisms, so that the investigation of reproductive patterns provides rich material on adaptation of life histories to local conditions (Wells 2007).

The great diversity of reproductive modes occuring in anurans (Haddad and Prado 2005) results in a considerable interespecific variation in temporal patterns of reproductive activity and in measurements of reproductive investment, such as egg size, clutch size and reproductive effort (Prado and Haddad 2005). While the interespecific variation in reproductive traits may also have an important phylogenetic effect, the intraspecific variation in reproductive traits is thought to be related with adaptations to local conditions (Wells 2007). Therefore, a reproductive strategy includes a combination of physiological, morphological and behavioral traits acting together to produce the optimum number of offspring under local environmental conditions (Duellman and Trueb 1994). An important component of a reproductive strategy, which allows the costs of reproduction to be measured, is the reproductive effort (Stearns 1992). For instance, the proportion of resources available to an organism which is invested in reproduction can be measured by establishing the proportions of gonad mass relative to body mass for individuals in a given population (e.g., Prado et al. 2000; Prado and Haddad 2005; Kaefer et al. 2007).

Considering that the use of an available resource for a given activity makes that resource unavailable to be used for another activities, negative relationships between two life history traits may occur, known as life history "trade-offs", where an increase in one trait is related to a decrease in the other. Regarding the costs of reproduction, two trade-offs have been long studied in animals (e.g., Stearns 1989). One refers to the trade-off between “age *vs.* size at maturity”, where by increasing the allocation of energy resources for reproduction, an individual will tend to decrease its rate of body growth (Stearns 1989). The other trade-off refers to the “reproductive investment *vs.* survival”, where an increase in the reproductive investment will imply in a tendency for a decreasing in survival rates due to a higher exposure to predators, for example (e.g., Tuttle and Ryan 1981). Body size is considered a key aspect of an organism life history because it is often correlated with measurements of resource use and reproductive investment (Roff 1992). For anurans, it has been suggested that body mass or body volume are better measures to investigate the effects of body size on reproductive investment than is body length, given that body mass or volume gives a more accurate measure of the frog’s internal cavity (Prado et al. 2000; Prado and Haddad 2005) and, in addition, because these measures are often positively correlated with body length (e.g., Caldart et al. 2012).

Ecological differences between sexes (e.g., in resource use, territoriality, fecundity and reproductive patterns) may lead to different ecomorphological adaptations in males and females due to niche divergence or due to sexual selection in traits that increase mating oportunities, such as body size in males of aggressive, territorial species. Sexual dimorphism in size (SSD) have been long studied for anurans (Shine 1979; Monnet and Cherry 2002; Kupfer 2007; Han and Fu 2013), leading to the general assumption that selection are expected to favor larger females because of the fecundity advantage associated with increased body size (Prado et al. 2000; Thomé and Brasileiro 2007) and favor larger males in species exhibitinh territoriality and physical combats (Shine 1989; Hudson and Fu 2013). Thus, one could expect male-biased SSD in species where male-male competition is a strong selective pressure and female-biased SSD in species where selective pressures related with increased fecundity are strong. However, as argued in a recent study on the evolution of SSD in anurans (Nali et al. 2014), SSD is also affected by size-dependent mechanisms acting on male and females. For instance, the fecundity advantage related with female body size may not be linear, i.e., females of small species may benefit more with increased body size than females of intermediate and large species (Nali et al. 2014). Besides, females had significant positive size-fecundity relationships regardless of their temporal pattern of reproduction (explosive or prolonged), whereas for males, territoriality was not significantly correlated with temporal breeding patterns nor correlated with SSD (Nali et al. 2014). Moreover, given that both sexes may present morphological fitness-related traits other than body size, sexual dimorphism within a given species should be investigated for additional morphological characters that may have evolved due to niche divergence or sexual selection, such as mouth size, snout shape, digital spines, limbs and foot characteristics and skin glands (e.g., Hudson and Fu 2013; Starnberger et al. 2013; Brunetti et al. 2014).

The Neotropical genera *Hylodes* and *Crossodactylus* (Hylodidae) present remarkable reproductive adaptations which have evolved due to their diurnal activity associated to acoustically complex habitats like torrent streams, such as the use of high-pitched calls, visual and multimodal signals (e.g., Haddad and Giaretta 1999; Lingnau and Bastos 2007; Caldart et al. 2014). However, little is known about basic life history aspects linked with reproduction in this family, such as seasonal variations in gametogenic cycles, size-fecundity relationships and sexual dimorphism. Therefore, case studies with local populations and individual species may provide valuable information on the evolution of reproductive traits and reproductive patterns, because these characteristic are affected by local conditions (Wells 2007) and may depend on the species in question and its mating system (Hudson and Fu 2013).

To date, studies with a few species of *Hylodes* and *Crossodactylus* indicate that territorial males are engaged in calling activity throughout the year in tropical (Hatano et al. 2002; Almeida-Gomes et al. 2007) and subtropical areas of the Atlantic Forest of Brazil (Caldart, unpublished data), but none study have yet investigated the reproduction patterns of these taxa through a histological approach. *Crossodactylus schmidti* is one of the few species of Hylodidae that presents a subtropical distribution in the Neotropical region, reaching the southernmost portion of Brazil in western Paraná, Santa Catarina and northern Rio Grande do Sul, up to Misiones, Argentina, and southeastern Paraguay (Frost 2015; Segalla et al. 2014). *Crossodactylus schmidti* has a predominant diurnal activity and inhabits rocky streams in which territorial males call (Figure 1) and perform visual and audiovisual signals upon rocks (Caldart et al. 2014). Here we investigated the reproductive biology of the diurnal stream frog *C. schmidti* in order to address the following aims: *1)* to describe the temporal pattern of reproduction through histological analysis of testis and ovaries; *2)* to investigate size-fecundity relationships and reproductive investment for both sexes; and *3)* to test mature individuals for sexual dimorphism in external morphological traits. Additionaly, we discuss the mating system of *C. schmidti* based on its reproductive patterns and behaviors.

2. Material and Methods

2.1. Study area and data acquisition

Specimens used in this study were collected in several streams within the Turvo State Park (27°14'34.08"S, 53°57'13.74"W, 376 m.a.s.l.), located in the municipality of Derrubadas, northwest region of the state of Rio Grande do Sul, Brazil. The park is one of the last large remnants of mesophytic semideciduous forest (*sensu* Oliveira-Filho et al. 2006) in southern Brazil, covering an area of 17,491.4 ha adjacent to the border of the Uruguay River and near the Argentinean forests of the Moconá Provincial Park and the Yabotí International Biosphere Reserve (SEMA 2005). Local climate is characterized as subtropical sub-humid with dry summer, has an annual rainfall of 1,787 mm, a mean annual temperature of 18.8 ºC and a mean temperature of the coldest month of 13.3 °C (ST SB v climate type, *sensu* Maluf 2000). Rainfall is evenly distributed troughout the year, summer occurs from December to March and winter from June to September (Maluf 2000).

Specimens were manually collected between 2009–2012 in a series of independent studies (licenses: SEMA #302/2009, #133/2010, #23/2011; ICMBio-IBAMA #18320–1, #29505-1), comprising a four-year temporal series with individuals of both sexes collected in all months (n = 137; 91 females and 46 males). Individuals were killed with 10% xylocaine immediately after collection, fixed in 10% formalin, preserved in 80% alcohol, and then housed in the Herpetological Collection of the Universidade Federal de Santa Maria (ZUFSM, Appendix 1). After preservation, individuals were dissected through ventral median incision and the ovaries and testes were extracted and individually preserved in 80% alcohol solution.

To investigate size-fecundity relationships and reproductive investment, measurements of testis and ovaries were obtained. Length and width of both testis were measured with a digital caliper to the nearest 0.01 mm, and testis mass was obtained with a precision scale to the nearest 0.0001 g. We then estimated the testicular volume for both testes through the formula of an ovoid spheroid: $V = 4/3\ \pi\ (L/2)\ (W/2)^2$, where L corresponds to the greatest length and W to the largest width of the testis. For females we measured ovary mass with a precision scale to the nearest 0.0001. We calculated for both sexes a gonadosomatic index (GSI) as a measure of the reproductive investment, as follows: GSI = GM x 100/BM, where GM and BM represent the gonad mass and body mass, respectively (see Kaefer et al 2007). For males, we consider gonad mass as the total mass of both testes.

After this procedure, ovaries and the right testis of all individuals were sent to the histological routine for dehydration in an increasing alcohol series (80% - 99% alcohol) and embedded in methacrylate glycol resin (LEICA Historesin Embedding Kit). From this material, slits of 3-4 µm were obtained on a rotary microtome (LEICA RM2245) to subsequent coloration with haematoxylin and eosin. For histological examination, the slides were observed in light microscopy (ZEISS PrimoStar with AxioCam ERc5s) and analyzed through the software ZEN LITE.

Spermatogenic stages were defined according to Burgos and Fawcett (1956), as follows: primary spermatogonia (SPG-I), secondary spermatogonia (SPG-II), primary spermatocyte (SPC-I), secondary spermatocyte (SPC-II), early spermatids (SPT-I), intermediate (elongated) spermatids (SPT-II), final spermatids (SPT-III), bundle of spermatozoa (SPZb), and free spermatozoa (SPZ). Males with free spermatozoa in the seminiferous tubules were considered as reproductive individuals. The stages of the ovarian cycle, in turn, were defined according to Oliveira and Santos (2004), as follows: primary follicle (FL-I), secondary follicles (FL-II), tertiary follicle (FL-III) and mature follicle (FL-IV); the presence of mature follicles was used to define reproductive females.

Last, to investigate sexual dimorphism we measured for each mature individual, with a digital caliper to nearest 0.01 mm and a digital scale to the nearest 0.01 g, the following external morphological traits: snout-vent length (SVL), mouth width (MW), head width (HW), head length (HL), eye diameter (ED), eye diameter-head length ratio (ED/HL), interorbital distance (IOD), internostril distance (IND), eye-nostril distance (END), tympanum diameter (TD), thigh length (THL), tibia length (TBL), foot length (FL), number of spines in the forelimb left toe (SPN-L), number of spines in the forelimb right toe (SPN-R) and body mass (BM). All variables were measured in the laboratory after collection, with the exception of BM, which was measured immediately after the capture of individuals in the field.

2.2. *Statistical analysis*

To investigate for seasonal variation in snout-vent length (SVL), body mass (BM) and in the gonadosomatic index (GSI) for males and females, we used one-way ANOVAs, grouping individuals according to seasons. To investigate size-fecundity relationships for both sexes we used linear regressions using only individual net body mass (body mass – gonad mass) as the predictor variable in regressions, because SVL and BM were strongly correlated for males (r = 0.84, *P* <0.0001) and females (r = 0.85, *P* <0.0001). Therefore, BM of males was regressed against testicular mass and testicular volume (total mass and total volume of both testes), and BM of females was regressed against ovarian mass. Correlations between body size and the values of GSI were tested through a Pearson correlation coefficient for males, and through a Spearman coefficient for females because of the non normal data distribution.

To test for sexual dimorphism in external morphological traits of adult individuals, we first investigated difference in SVL between males and females through a T test. Then we analyzed Pearson correlation coefficients between morphological traits, and excluded HW, HL, ED/HL, and TBL because they were strongly correlated with other morphological traits (r >0.6 and *P* <0.05 in all cases). Thus, we investigated differences in BM, MW, ED, IOD, IND, END, TD, THL and FL through one-way ANCOVAs using SVL as the covariable, given that SVL was also positively correlated with all other traits (r >0.5 and *P* <0.05 in all cases). Last, we test for sexual differences with Mann-Whitney tests in secondary sexual traits such as number of spines in the forelimbs toes (SPN-L and SPN-R).

In all statistical analysis we use only mature individuals for which we have the body mass measured in the field (62 from 69 mature females; 41 from 44 mature males). All data were tested for normality and for homogeneity of variances before performing statistical analyses, and were log-transformed if necessary. Data and residuals used in parametric tests fitted a normal distribution. Analyses were made in the software Past, version 2.17c (Hammer et al. 2001) and in R software v. 2.12.2 (R Development Core Team 2011) through the Vegan package (Oksanen et al. 2009), with alpha set as significant when *P* <0.05.

3. Results

3.1. Temporal pattern of reproduction

Histological analyses of ovaries of females (n = 91) and testes of males (n = 46) (Figure S1-S2) revealed that mature individuals of both sexes occurred in all months. Free spermatozoa, sperm bundles and germ cell cysts in several maturity stages were found in testes of all males with the exception of two individuals collected on February and March (n = 44, 96%; Figure 2-A), with 42 (91%) of them showing all nine stages of the spermatogenesis cycle. For females, ovaries of most individuals presented the fourth follicle stage (n = 69, 76%) or all four follicle stages (n = 65, 71%; Figure 2-B). The proportion of mature males, in relation to our monthly sample size, varied from 75% in February and 67% in March, to 100% in the remaining months (Figure 2-C). For females, the proportion varied from a minimum of 50% of mature individuals in February and August to a maximum of 100% in September, with intermediate proportions of mature females in the remaining months (Figure 2-C). Despite a tendency of a decrease in body size of individuals in the winter, there was no significant seasonal variation neither for males nor for females in snout-vent length (ANOVA: males, $F_{3,37} = 2.74$, $P = 0.06$; females, $F_{3,58} = 1.45$, $P = 0.24$), body mass (ANOVA: males, $F_{3,37} = 1.44$, $P = 0.25$; females, $F_{3,58} = 1.03$, $P = 0.39$) or in the gonadosomatic index (ANOVA: males, $F_{3,37} = 1.80$, $P = 0.16$; females, $F_{3,58} = 0.93$, $P = 0.43$) (Table 1, Figure 3). In addition, we collected free-swimming tadpoles varying from the stages 26 to 39 throughout the year, and registered males in calling activity throughout the year as well.

3.2. Size-fecundity relationships

Overall, body mass had a significant effect on gonad mass for both sexes (Figure 4). Testicular mass had a positive relationship with body mass ($R^2_{adj} = 0.27$, $F_{1,39} = 14.32$, $P < 0.001$), whereas testicular volume had a marginally significant relationship with body mass, though with a low coefficient of determination ($R^2_{adj} = 0.09$, $F_{1,39} = 3.70$, $P = 0.06$). For females, in turn, the effect of body mass was much stronger, with larger females showing larger ovary mass ($R^2_{adj} = 0.56$, $F_{1,61} = 78.81$, $P < 0.001$).

Most males (n = 24 from 41) and females (n = 36 from 62) presented values of GSI lower than the mean GSI, though the frequency distribution of females was not normal, with a long tail of few females with high GSI values (Shapiro-Wilk, W = 0.914, $P < 0.05$), whilst for males the frequency distribution was normal and more evenly distributed around the mean (W = 0.961, $P > 0.05$) (Figure 5). The overall mean of the GSI for males was 0.92 ± 0.29 (range 0.35 – 1.44) and male SVL and reproductive investment were not correlated (r = 0.07, $P > 0.05$). Both the smallest and the largest mature males presented reproductive investments close to mean value found for males (smallest: SVL = 22.14, GSI = 0.79; largest: SVL = 27.95, GSI = 0.85). Besides, two males of very similar sizes presented the lowest (SVL = 23.34, GSI = 0.35) and the highest reproductive investments (SVL = 23.53, GSI = 1.44).

For females, the overall mean of the GSI was 4.89 ± 3.72 (range 0.14 – 16.72). There was a weak but significant positive correlation between female SVL and reproductive investment (rs = 0.42, *P* <0.001). However, with smaller and larger females analyzed separately and considering the overall mean of female SVL as a breakpoint, the correlation between SVL and reproductive investment was much stronger for smaller females (rs = 0.70, *P* <0.001), whilst not significant for larger females (rs = -0.10, *P* >0.05) (Figure 6). The smallest mature female also presented the lowest reproductive investment (SVL = 21.78, GSI = 0.14), while the largest female present a reproductive investment close to mean value of females (SVL = 31.68, GSI = 3.58). The highest reproductive investment among females was found for a medium-sized female (SVL = 27.29 mm, GSI = 16.72).

3.3. Sexual size dimorphism

Our results showed that the smallest mature female had a SVL of 21.78 mm, whereas the smallest mature male had a SVL of 22.14 mm. Considering only mature individuals, females were significantly larger than males in SVL (T test, t = -6.780, P < 0.0001), with a mean SVL of 27.68 mm (± 2.26; range 21.78 – 31.68), whereas males had a mean SVL of 25.03 mm (± 1.33; range 22.14 – 27.95) (Figure 7). After removing the effects of SVL on other external morphological traits, there was sexual dimorphism only in mouth width, with females having larger mouth width than males (ANCOVA, $F_{1,101}$ = 12.55, *P* <0.001) (Figure 7, Table 2). Besides, the number of spines in forelimb toes did not differ between sexes neither for the right (Mann-Whitney, *U* = 1042, *P* = 0.09) nor for the left forelimb toe (Mann-Whitney, *U* = 1093, *P* = 0.17). Males had on average 5 ± 1 spines in right and in left forelimb toes, whilst females had an average of 4 ± 1 spines in each forelimb toe.

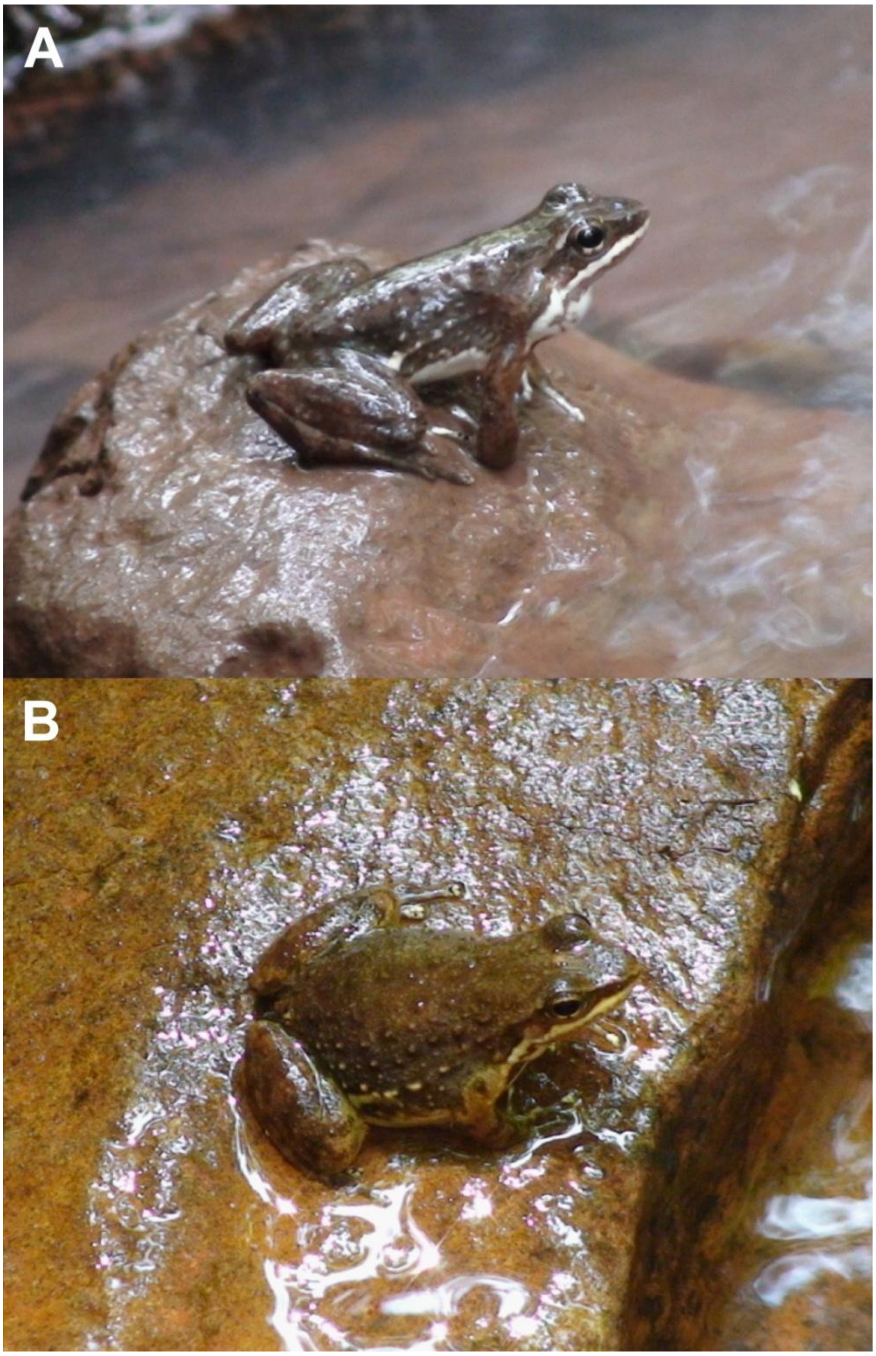

Figure 1. A male (A) *Crossodactylus schmidti* calling from its typical calling site and (B) an ovigerous female of *C. schmidti* from a stream within the Turvo State Park, Rio Grande do Sul state, southern Brazil.

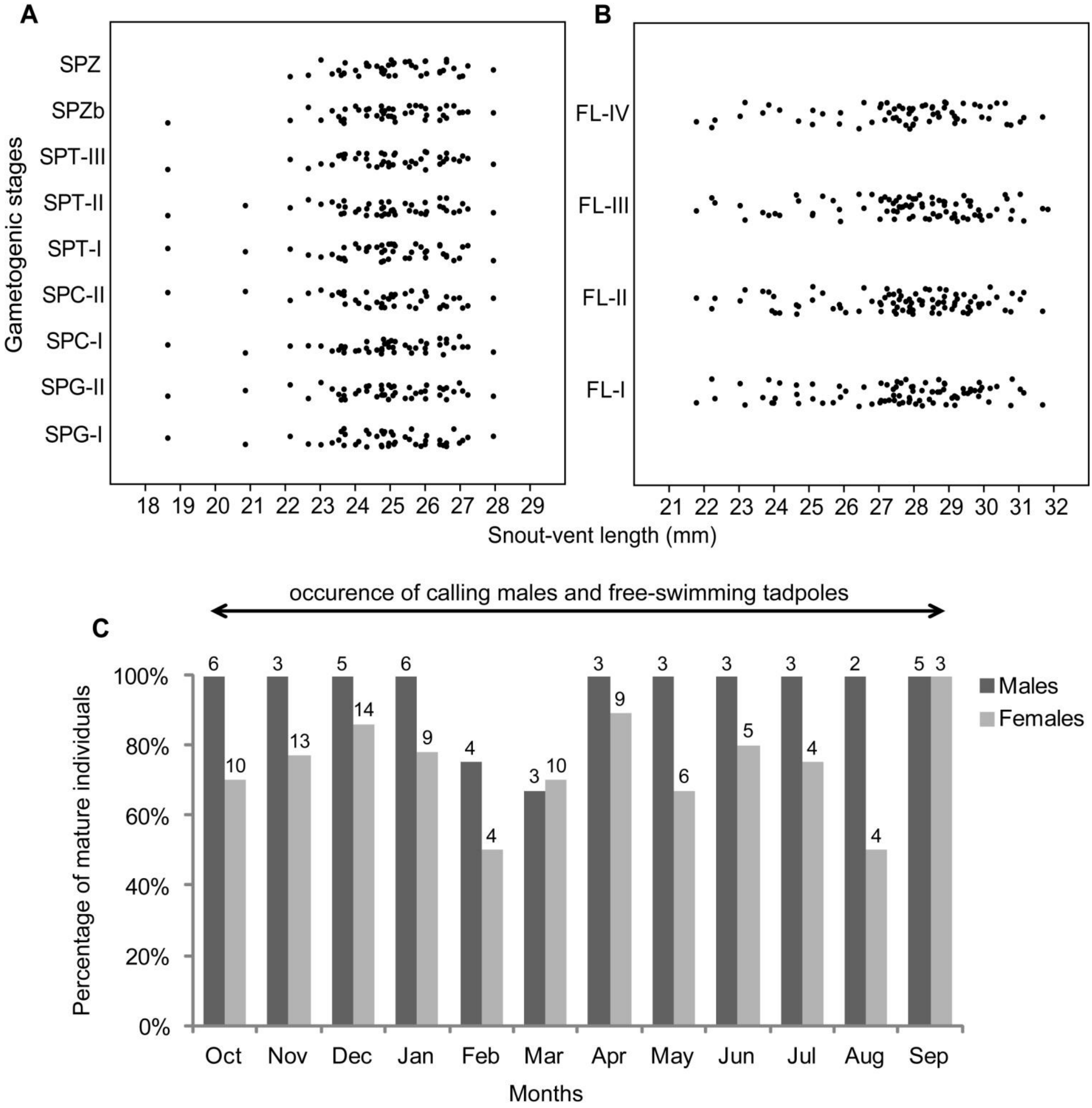

Figure 2. Distribution of gametogenic stages according to body size of (A) males and (B) females, and (C) monthly proportion of mature individuals of *Crossodactylus schmidti* from southern Brazil, collected between 2009–2012. Each dot represents one individual, though the same individual may present more than one or even all gametogenic stages. Values above each bar indicate the total number of individuals analyzed per month.

Table 1. Seasonal variation (mean ± standard deviation) in snout-vent length (SVL), body mass and in gonadosomatic index (GSI) for mature males (n = 41) and females (n = 62) of *Crossodactylus schmidti* from southern Brazil.

Sex	Season	SVL (mm)	Body mass (g)	GSI	n
Male	Spring	25.41 ± 1.21	1.99 ± 0.33	0.89 ± 0.26	16
	Summer	25.45 ± 1.20	1.96 ± 0.32	0.81 ± 0.31	11
	Autumn	24.41 ± 1.53	1.71 ± 0.37	1.07 ± 0.30	9
	Winter	24.00 ± 0.85	1.85 ± 0.42	1.04 ± 0.28	5
Female	Spring	27.95 ± 2.22	2.56 ± 0.52	5.67 ± 3.93	26
	Summer	28.27 ± 1.81	2.54 ± 0.44	3.66 ± 2.69	15
	Autumn	27.12 ± 2.62	2.46 ± 0.71	4.82 ± 4.29	16
	Winter	26.30 ± 2.09	2.10 ± 0.52	4.79 ± 3.31	5

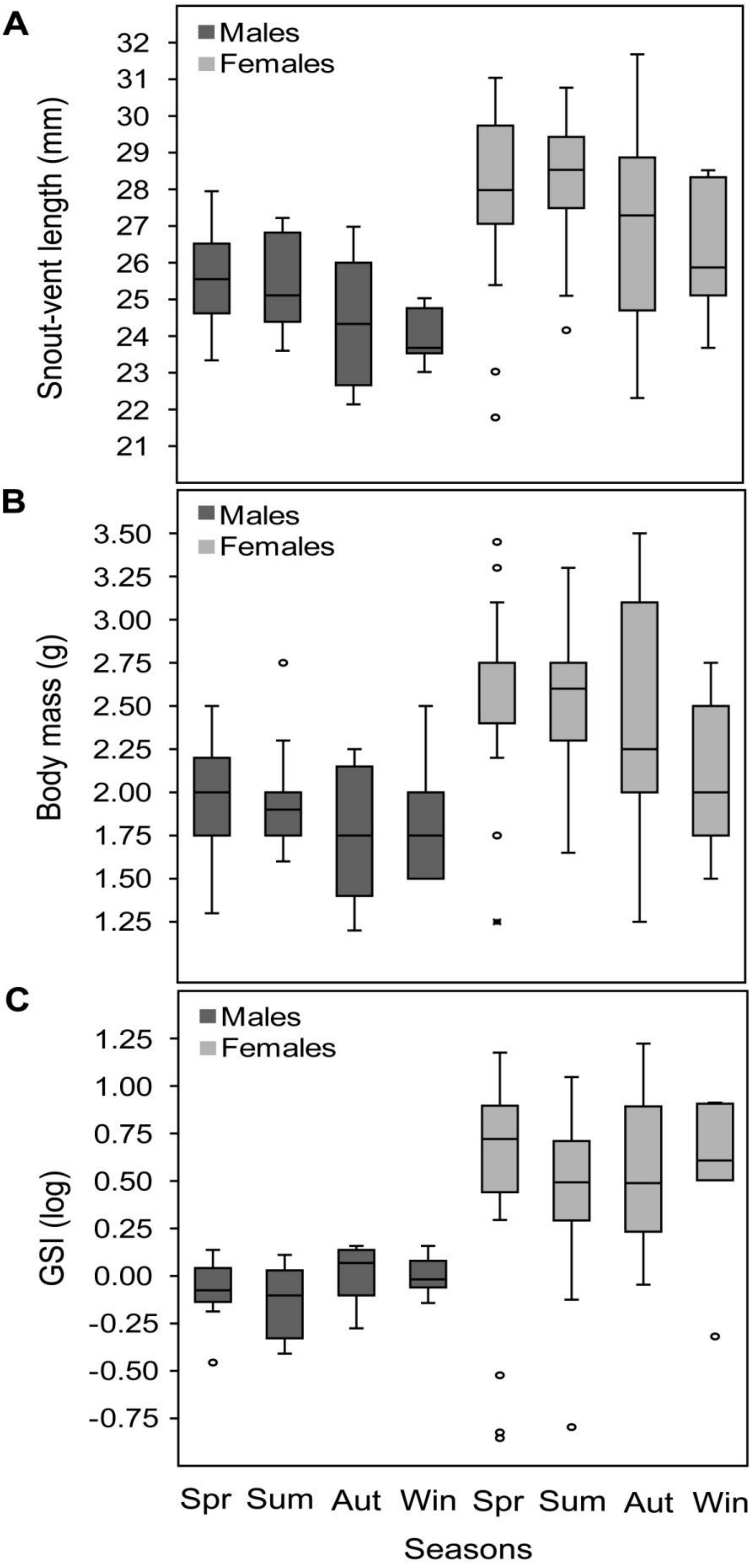

Figure 3. Seasonal variation in (A) snout-vent length (SVL in mm), (B) body mass (g) and (C) in gonadosomatic index (GSI) for males and females of *Crossodactylus schmidti* from southern Brazil.

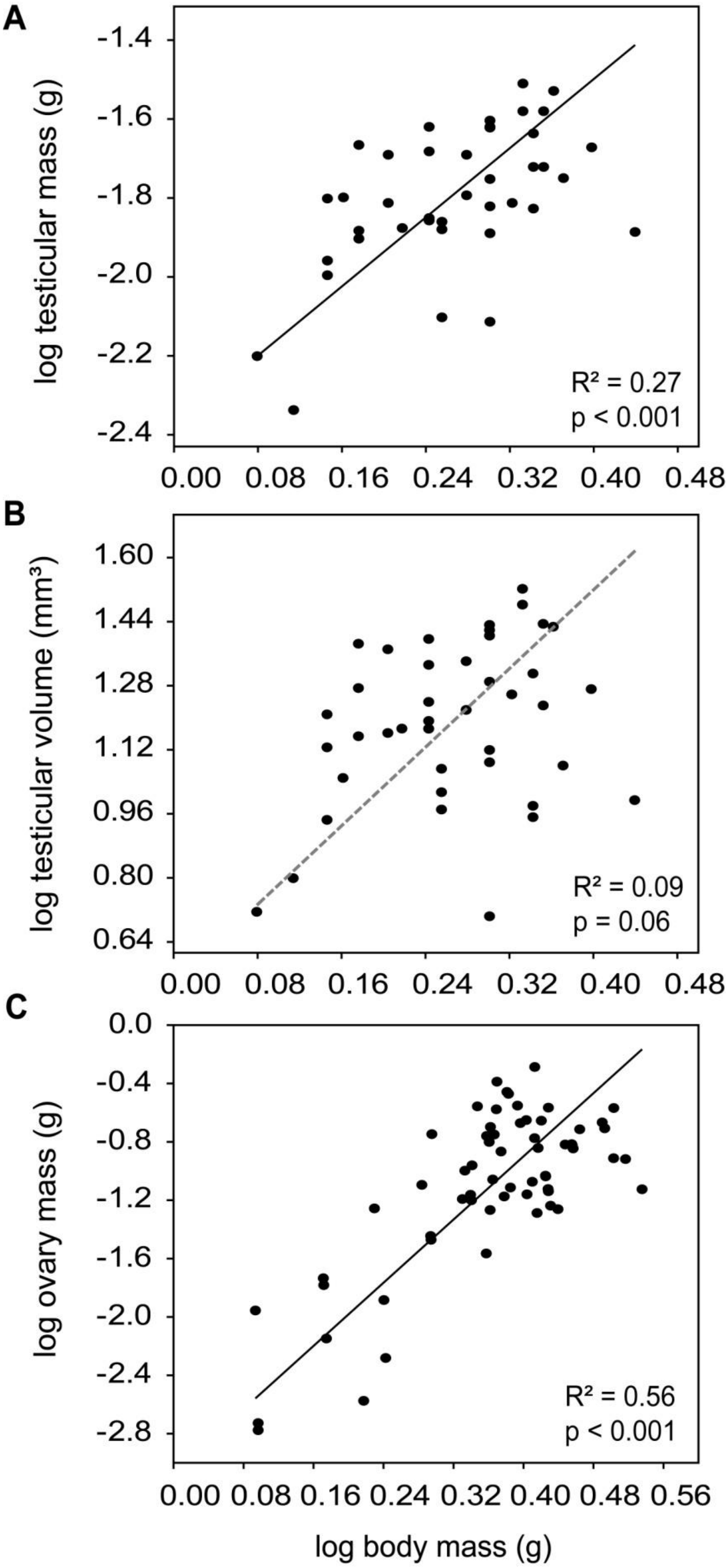

Figure 4. Relationship between (A) testicular mass and body mass, (B) testicular volume and body mass, and (C) ovary mass and body mass of mature individuals of *Crossodactylus schmidti* from southern Brazil. Solid regression lines represent significant results, and the dashed line represents a marginally significant result.

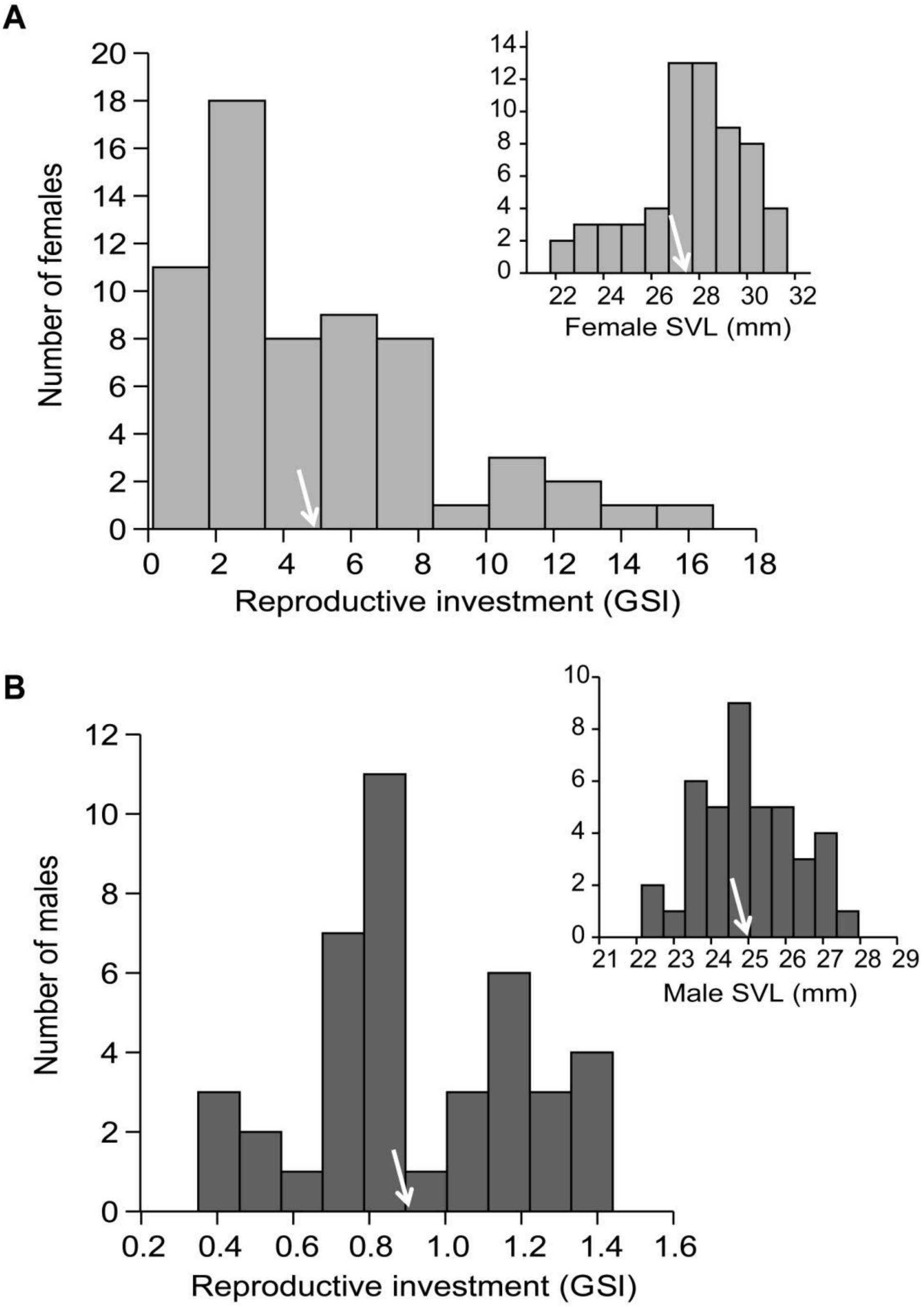

Figure 5. Frequency distribution of the reproductive investment (GSI, gonadosomatic index) and snout-vent length (SVL) of (A) mature females and (B) mature males of *Crossodactylus schmidti* from southern Brazil. White arrows indicate the mean value for each data set.

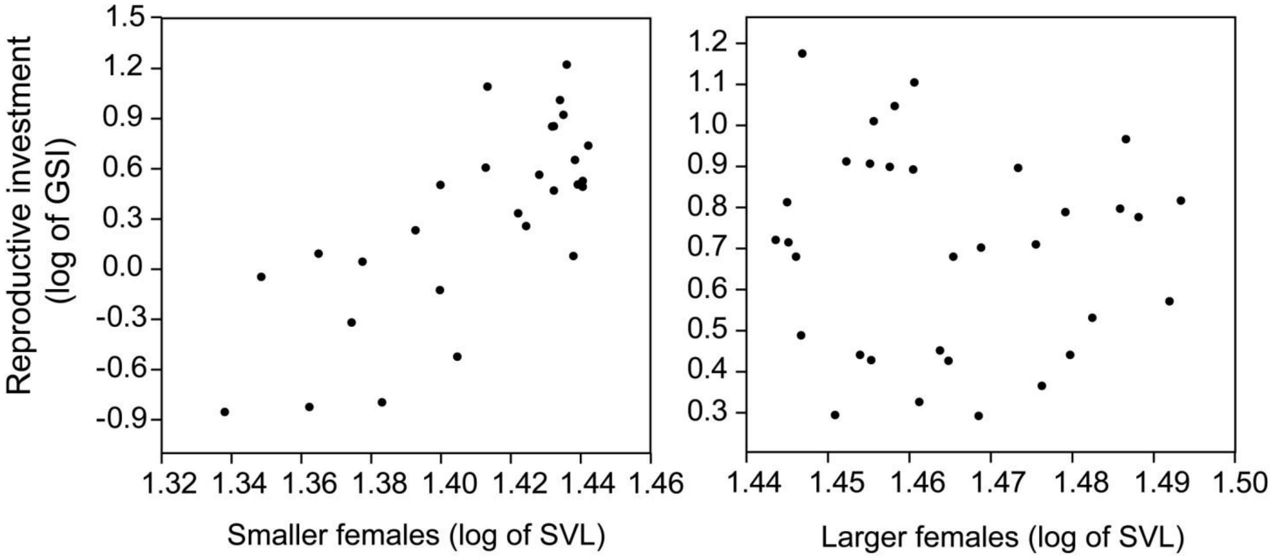

Figure 6. Dispersion diagrams from Spearman's correlations between snout-vent length (SVL) and reproductive investment (GSI, gonadosomatic index) of females of *Crossodactylus schmidti* smaller than the mean female SVL (rs = 0.70, *P* <0.001), and between SVL and reproductive investment of females larger than the mean female SVL (rs = -0.10, *P* >0.05).

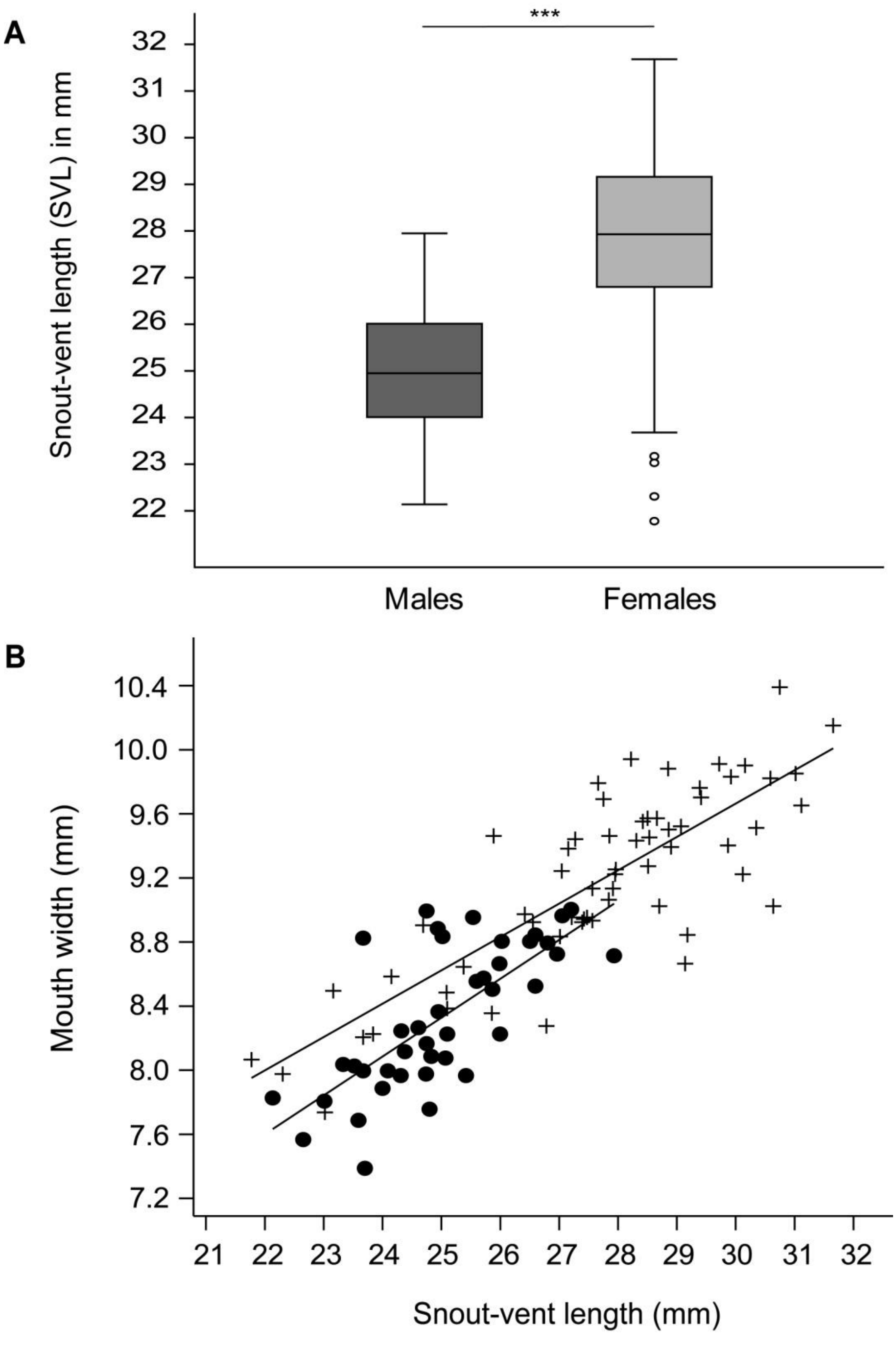

Figure 7. Sexual dimorphism in (A) snout-vent length (*** significant difference, T test: $t = -6.7805$, $P < 0.0001$) and in (B) mouth width of *Crossodactylus schmidti* from southern Brazil, with snout-vent length as covariable (black circles = males; crosses = females; ANCOVA: $F_{1,101} = 12.55$, $P < 0.001$).

Table 2. Standardized means ± standard deviation and ranges of morphological traits of mature individuals of *Crossodactylus schmidti* from southern Brazil, followed by statistical significances of the comparisons between the sexes, with snout-vent length as covariable of all other morpholocical traits (one-way ANCOVA). BM, body mass; MW, mouth width; ED, eye diameter; IOD, inter-orbital distance; IND, inter-nostril distance; END, eye-nostril distance; TD, tympanum diameter; THL, thigh length; FL, foot length. Bold value indicate significant difference (*P* <0.001).

Morphological trait (mm)	Males (n = 41)	Females (n = 62)	One-way Ancova			Homogeneity of slopes	
			F	df	*P*	F	*P*
BM	1.87 ± 0.35 (1.20 - 2.75)	2.49 ± 0.56 (1.25 - 3.50)	0.965	1, 101	0.3283	0.101	0.7515
MW	8.34 ± 0.45 (7.39 - 9.01)	9.19 ± 0.57 (7.74 - 10.40)	12.550	1, 101	**0.0006**	0.647	0.4232
ED	3.00 ± 0.24 (2.14 - 3.42)	3.21 ± 0.28 (2.50 - 3.97)	0.832	1, 101	0.3638	0.477	0.4916
IOD	5.26 ± 0.55 (2.74 - 5.89)	5.61 ± 0.36 (4.70 - 6.51)	1.170	1, 101	0.2821	0.799	0.3737
IND	2.86 ± 0.27 (2.45 - 3.64)	3.01 ± 0.27 (2.34 - 3.76)	0.000	1, 101	0.9824	0.344	0.5587
END	2.17 ± 0.24 (1.66 - 2.84)	2.36 ± 0.21 (1.70 - 2.98)	0.081	1, 101	0.7762	0.429	0.5138
TD	2.24 ± 0.21 (1.92 - 2.80)	2.29 ± 0.23 (1.83 - 2.95)	1.585	1, 101	0.2110	0.002	0.9647
THL	12.46 ± 0.70 (10.70 - 13.59)	13.34 ± 0.84 (11.21 - 14.88)	1.407	1, 101	0.2384	1.494	0.2245
FL	18.48 ± 1.46 (12.72 - 21.14)	19.79 ± 1.08 (17.18 - 21.76)	2.042	1, 101	0.1562	3.739	0.0560

4. Discussion

Our results indicate through different lines of evidence that the studied population of *Crossodactylus schmidti* has a continuous pattern of reproduction in subtropical Brazil. We found mature individuals of both sexes throughout the year, with the proportion of mature males per month varying from 67% to 100% in relation to our monthly sample, whilst for females it varied from 50% in February and August to 100% in September, with intermediate proportions in remaining months. This pattern is reinforced by the absence of a significant seasonal variation in body size, body mass and in the gonadosomatic index for both sexes, as is expected for species that show continuous reproduction (Callard et al. 1978). Therefore, the gametogenic cycle of *C. schmidti* fits into the continuous pattern according to the classifications of Lofts (1974) and Huang et al. (1997), with no interruption in the production of sperm and oocytes within the population along the year, though the proportions of mature individuals along the year varied much more for females. By opposite, species that reproduce seasonally show a significant reduction of gonads and no production of spermatozoa after mating periods (Ngo and Ngo 2013).

The continuous pattern of sperm production in *C. schmidti* is also consistent with the behavior of males, which were observed engaged in calling activity and in agonistic interactions year-round in the study area (Caldart, unpublished data). Field observations from other studies (Caldart et al. 2011; Caldart et al. 2014) suggests that the mating system of *C. schmidti* relies primarily into the resource defense polygyny type (*sensu* Emlen and Oring 1977), as territorial males defend calling sites near to adequate places to excavate, together with the female, the entrance of the subaquatic chamber where oviposition occurs (reproductive mode #3 *sensu* Haddad and Prado, 2005). The frequent male-male agonistic interactions (Caldart et al. 2014) suggest that males defend calling and oviposition sites due to the continuous presence of mature females, which is now confirmed by the histological analysis of gonads. Then, by means of the evidence of the histological analysis and behavioral observations, it is reasonable to assume that the continuous presence of mature females in the population plays a role in the existence of a resource defense polygyny mating system, given that subaquatic chambers are a crucial resource for reproduction and that the temporal availability of these sites may vary due to hydrological disturbance in the spatial dynamics of lothic habitats (Lake 2000). Furthermore, as the proportion of mature females varied along the year, dropping by half in some months, resource defense through agonistic interactions between males must influence access to mature females.

Last, we found free-swimming tadpoles of *C. schmidti* in all months (varying from the stages 26 to 39), which indicates that oviposition may occur throughout the year, as also observerd for *Crossodactylus gaudichaudii* in southeastern Brazil (Almeida-Gomes et al. 2012). However, a study on population dynamics is needed to address the issue of recruitment of juveniles, given that for the closely related torrent frog *Hylodes asper*, a seasonal pattern of population cycle with a high annual rate of juvenile recruitment was reported (Patto and Pie 2001). These authors reported an almost complete turnover of individuals within the population of *H. asper* during one year, where newly recruited juveniles grew rapidly during 8-10 months, were ready to reproduce within one year, and by the end of the year adults comprised almost the entire population. Although mature males and females, calling males and free-swimming tadpoles of *C. schmditi* were found throughout the year, whether there is an overlap of generations along the year still needs to be investigated.

Size-fecundity relationships and reproductive investment may vary among anuran species from subtropical areas, but in general there is a positive relationship between body size and gonad size (e.g., Melchiors et al 2004; Camargo et al 2005; Maneyro et al 2008) and a major reproductive investment in gonads by females than by males (e.g., Halliday and Tejedo 1995). For *C. schmidti*, the relationship between body mass and gonad mass was significant for both sexes, but was stronger for females. This is expected according to the fecundity advantage hypothesis, which postulates that selection will favor large females due to the adaptive advantage of a greater egg production associated with increased body size (Shine 1989). This advantage seems to be strongest for females of small-bodied species – as is the case of *C. schmidti* – than for females of intermediate or large species, regardless of the temporal pattern of reproduction (Nali et al. 2014).

Our results also suggest that larger females have an advantage in relation to smaller females, given that SVL and reproductive investment were correlated; however, this correlation was not strong (rs = 0.42, P <0.001), possibly because most of the females in our sample were larger than the mean SVL of females. Thus, if larger females had an effect in the low correlation coefficient, when analyzing separately females smaller and larger than the average female SVL, there should be a significant increasing trend between SVL and reproductive investment for the former, and a not significant relationship for the latter; we corroborate this prediction as the coefficient increased for smaller females (rs = 0.70, P <0.001) and decreased for larger females (rs = -0.10, P >0.05). This suggests that females of *C. schmidti* may face a life history trade-off related with the costs of reproduction. For instance, by allocating a major investment of the energetic resources into gonad development, smaller females may have their growth rate reduced (Roff 1992; Stearns 1992). On the other hand, larger females had low correlation between SVL and reproductive investment likely due to an effect of age or temporal partition of mating opportunities, since if oviposition sites defended by males vary in quality, females may have successive mating opportunities with different quality males or, alternatively, may delay ovulation until a favorable site is found (Wells 1977).

Although for anuran species fecundity can be a plastic trait that may change due to environmental conditions (e.g., Elmberg 1991; Kaplan and King 1997), the lack of seasonal differences in reproductive investment for both males and females – despite of the local climatic seasonality – indicates strong intrasexual competition for mates and reproduction-related resources. Competition must be strong specially between males, as the proportion of mature females varied much more along the year than did the proportion of mature males, and because females may have ample opportunities to compare the quality of potential mates in prolonged-breeding anurans that defend resource-based territories (Wells 2007). The facts that smaller and larger males had similar reproductive investments and, conversely, that similar sized males presented the lowest and the highest reproductive investments, support the strong male-male competition argument, and also explain why males are aggressive toward juveniles (Caldart et al. 2014), as we found that males may reach sexual maturity at small sizes.

Regarding sexual dimorphism, we suggest that the strong size-fecundity relationship in females of *C. schmidti* has played a role in the evolution of a female-biased sexual dimorphism in snout-vent length. As discussed above, the advantage of increased body size must be an important pressure on female body size (Nali et al. 2014), and since body mass and snout-vent length are strongly correlated, we expect this pressure to be affecting both traits. One could also expect males of *C. schmidti* to be larger than females, due to their aggressiveness and territorial behavior which often leads to intense agonistic interactions. However, male-biased sexual size dimorphism is much less common in amphibians, having evolved mainly in species where male-male combats involves weapon-like body structures that may injury the opponent (Shine 1979; Hudson and Fu 2013) and in species where parental care is an important life history trait (Han and Fu 2013). Neither case fits in the case of *C. schmidti* males, which engage in escalated, wrestling-mediated combats and do not seem to show paternal care.

There was also sexual dimorphism in the mouth width of *C. schmidti*. Sexual differences in mouth parts are thought to be the result of divergence in the trophic niche or, alternatively, to adaptations not directly related to feeding habits, e.g., the use of mouth parts to excavation or to nest building (Shine 1989). In the light of the available evidence for *C. schmidti*, we believe that the sexual dimorphism in mouth width is likely a result of sexual divergence in prey consumption. For example, among the many prey items consumed by *C. schmidti*, larvae of Lepidoptera was one the most volumetrically important item and, indeed, females tended to consume more larvae of Lepidoptera than males (Caldart et al. 2012). Besides, it has been suggested that since females do not spend much time in defending territories and engaging in agonistic interactions, they would be able to forage and feed more frequently than males to sustain the energetic requirements associated with egg production (Donnelly 1991; Valderrama-Vernaza et al. 2009; Caldart et al. 2012). Last, the absence of sexual dimorphism in the spines of forelimb toes suggests that these structures may play a role in mutual tactile interactions during courtship. This notion is supported by observations of a male positioning himself right in front of his mate when faced to an intruder male prior to oviposition, and in such cases the female seems to tactilely interact with her mate through an amplexus-like tactile stimulus (Caldart, personal communication; Appendix A). Further investigations and descriptions of tactile behaviors will be helpful to understand the role of digital spines in social interactions.

In conclusion, our results indicate that the studied population of *C. schmidti* has a continuous pattern of reproduction in subtropical Brazil, with gametogenesis, calling activity and free-swimming tadpoles occurring year-round. We presented histological evidence that support the existence of intrasexual competition, since *1)* both males and females may reach sexual maturity at small sizes, *2)* mature individuals of both sexes are available in all months, with the proportion of mature females varying more along the year than the proportion of mature males, *3)* size-fecundity relationships were positive for both sexes, but stronger for females, and *4)* males continuously compete for territories suitable for calling for mates and for oviposition. Thus, we believe that larger individuals must have an advantage for reproduction, because larger males should be benefited at establishing and defending calling and oviposition sites in a resource defense polygyny mating system, whilst larger females may potentially be benefited by having a larger spawn when compared to smaller females. However, studies evaluating male mating success regarding territorial defense and paternity are needed to confirm the resource defense polygyny mating system in *C. schmidti*.

Acknowledgments

We are grateful to Cynthia Prado, Camila Both, Selvino Neckel de Oliveira and Sandro Santos for valuable suggestions that greatly improved this study, to the staff of the 'Laboratório de Herpetologia – UFSM' for their assistance in field activities, and to Tiago Theis for his valuable help in the preparation of slides. V.M.C. thanks CAPES for the doctoral fellowships and S.Z.C. is grateful to CNPq for the award of a research fellowship (No. 304929/2012-3). Collection of individuals at Turvo State Park was performed in accordance with the licenses issued by SEMA-RS (#302/2009, #133/2010 and #23/2011) and by ICMBio-IBAMA (#18320–1 and #29505-1).

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Appendix 1. Voucher specimens of *Crossodactylus schmidti* analyzed in this study (n = 137), housed in the Herpetological Collection from Universidade Federal de Santa Maria (ZUFSM).

ZUFSM 4539, 4643-4644, 4646-4647, 4649-4651, 4668-4670, 4672-4676, 4678, 4680-4684, 4687, 4689-4692, 4695-4696, 4860-4861, 5196-5242, 5252-5265, 5267, 5269, 5780-5782, 6106-6144, 6147.

Supplementary material

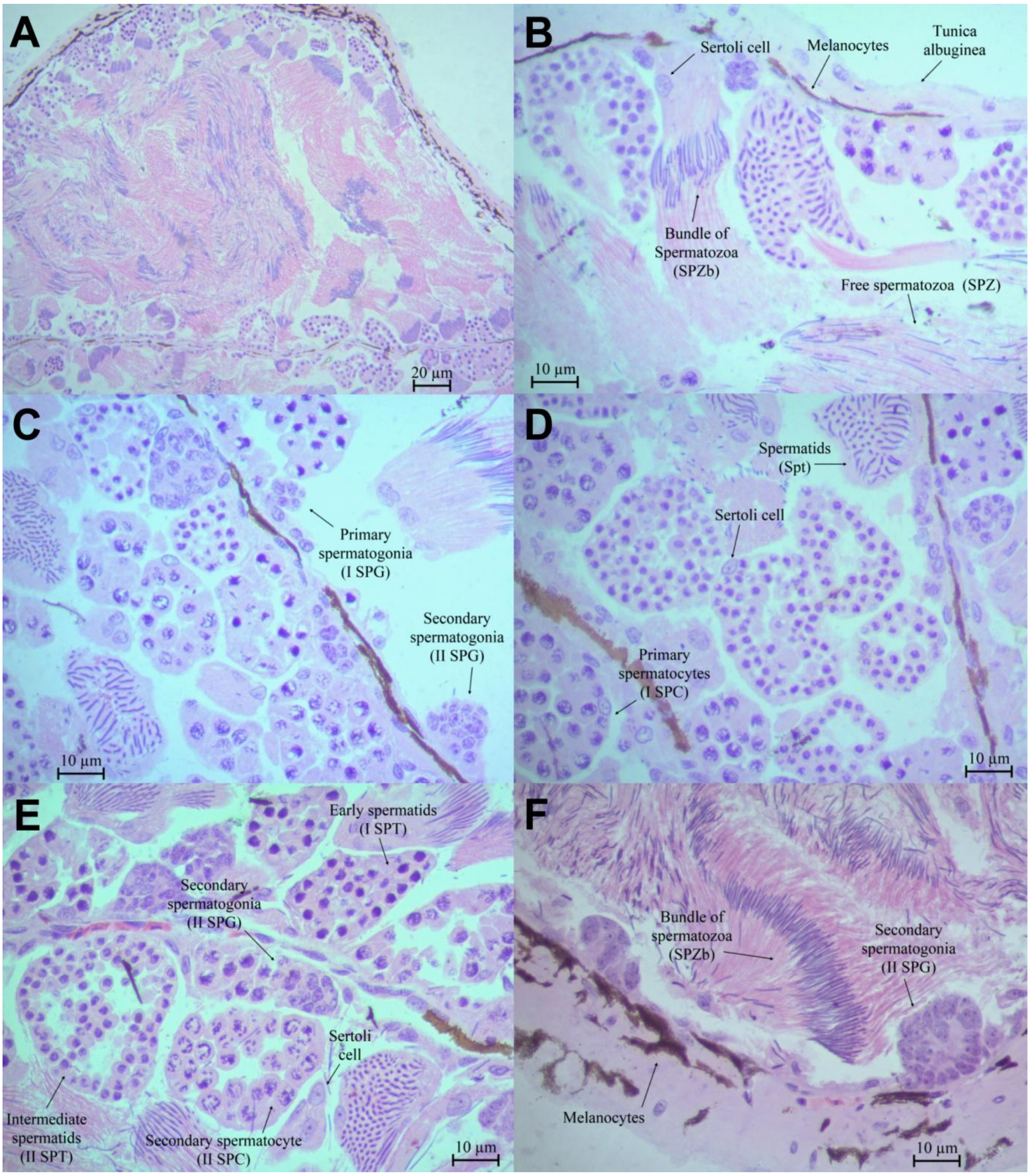

Figure S1. Histological slides of testis of *Crossodactylus schmidti* from southern Brazil. A) Seminiferous tubule; B–F) Several maturation stages of the spermatozoa development cycle.

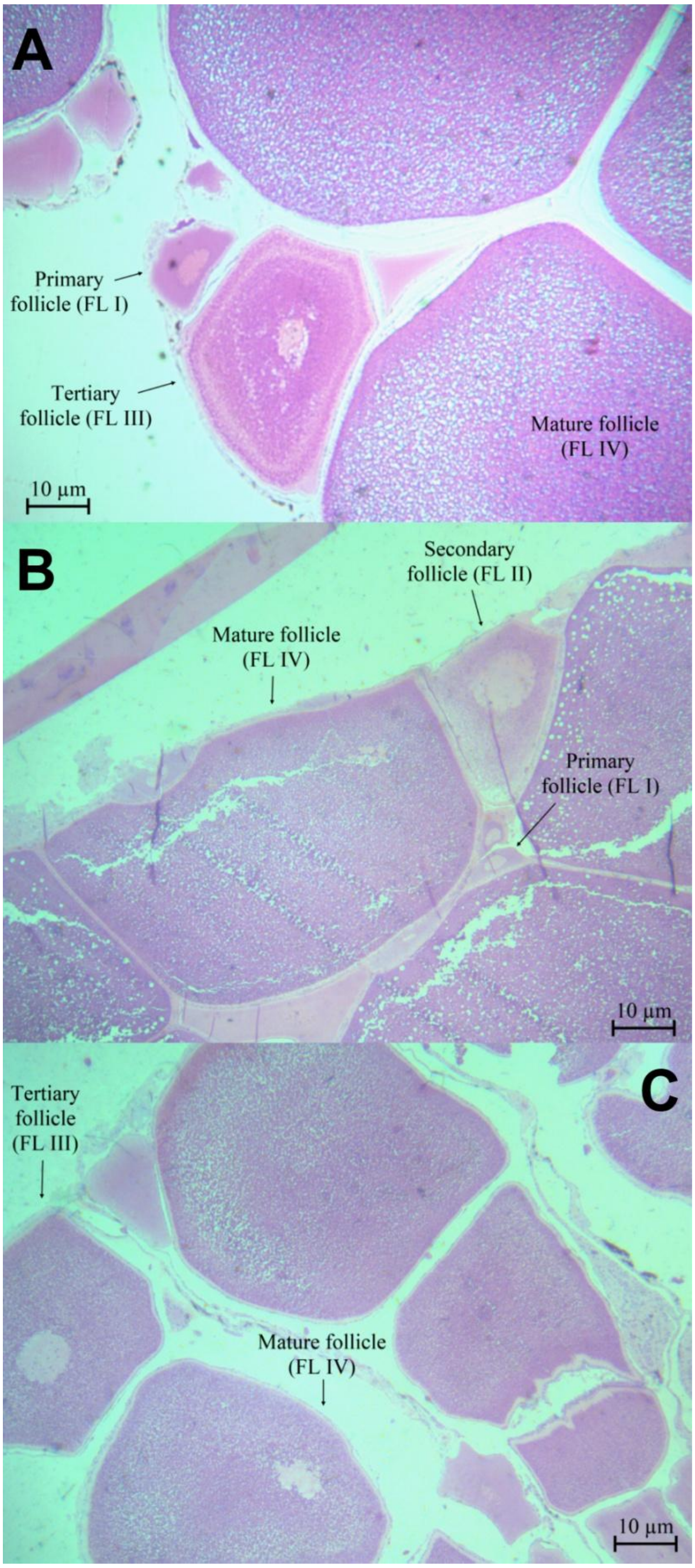

Figure S2. Histological slides of ovaries of *Crossodactylus schmidti* from southern Brazil. A–C) Several maturation stages of the oocytes development cycle.

CAPÍTULO V

Parasitic flies attracted to frog calls in the Austral Neotropics: evidence of the interaction and discussion on potential selective pressure

Parasitic flies attracted to frog calls in the Austral Neotropics: evidence of the interaction and discussion on potential selective pressure

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Abstract. The interaction between eavesdroppers and signalers has been investigated for a wide range of organisms, and though many flies may parasitize anurans, the cue used to detect frog hosts is not known for some taxa. Besides, the interaction between parasitic flies and anurans has been poorly addressed in the austral Neotropics. Aiming to address which taxa of parasitic flies are acoustically-oriented toward frog calls, we investigate this interaction at an area of seasonal forest in southern Brazil. We broadcasted calls of three species from the study area: *Crossodactylus schmidti* (Hylodidae), *Physalaemus* cf. *gracilis* (Leptodactylidae) and *Scinax perereca* (Hylidae). For each species we created a playback loop based on their mean call traits, and we also created a white noise loop to compare the capture patterns between taxa. From 139 sessions (~105 hours) we collected flies of the genera *Corethrella* (Corethrellidae, n=188), *Forcipomyia* (Ceratopogonidae, n=80) and *Uranotaenia* (Culicidae, n=16). Number of *Corethrella* flies significantly differed between acoustic and silent traps, with frog species analyzed altogether or separately. In contrast, number of specimens of *Forcipomyia* and *Uranotaenia* did not differ between acoustic and silent traps, neither with frog species altogether or separately. In addition, a comparison between traps broadcasting a white noise and silent traps showed that proportions of flies did not differ for any of the diptera taxa, confirming that *Corethrella* was attracted to frog calls, whilst *Forcipomyia* and *Uranotaenia* were captured by chance. By considering different taxa of parasitic flies and by using a white noise stimulus, we demonstrated that *Corethrella* was the only taxon of parasitic flies clearly attracted to calls of *C. schmidti*, *P.* cf. *gracilis* and *S. perereca*, a pattern reinforced by the lack of differences between the white noise stimulus and silent traps. The potential selective pressure of *Corethrella* flies on male frogs is discussed. This is the first study to investigate such interaction in the austral Neotropics using different phonotaxis treatments.

Keywords: acoustic communication, eavesdroppers, *Corethrella*, parasitism, anurans, white noise

1. Introduction

An illegitimate receiver is a term used to refer to the non-target audience of a signal emitted by a given animal. Non-target animals may exploit other animals' signals in their advantage, then referred to as eavesdroppers, and thus imply potential adaptive costs to the signaler (Zuk and Kolluru 1998; Peake 2005). In the perspective of the animal having their signals exploited, the different effects that an eavesdropper may imply will depend on the nature of the ecological relationship between the signaler and the eavesdropper exploiting its signals. For instance, an illegitimate receiver of a given acoustic signal could be a non-target conspecific (Halfwerk et al. 2014), a predator (Tuttle and Ryan 1981), a parasitoid (Zuk et al. 2006) or a parasite (Bernal et al. 2006; Grafe et al. 2008).

In cases where the eavesdropper is a predator or a parasite, rapid evolutionary changes may occur and novel behavioral strategies may appear. Good examples of the selective pressure of acoustically-oriented eavesdroppers come from the well known interaction between frog-eating bats and male túngara-frogs (Tuttle and Ryan 1981), in which male frogs evolve the primary strategy of cease calling when bats are nearby, because bats may also echolocate the water-borne ripples generated by the vocal-sac movements of frogs (Halfwerk et al. 2014), and from the interaction between parasitoid flies and crickets in Hawaii, where in a few years after the introduction of a parasitoid fly, male crickets lacking a typical stridulatory structure on wings used to produce their mating calls became much more abundant than males having such structure (Zuk et al. 2006). In both interactions the selective pressure of the eavesdroppers implies a cost in the signalers reproductive success because their acoustic signals are sexually selected traits (Ryan 1980; Zuk et al. 2006).

In cases where the eavesdropper is a parasite, despite the non-deadly interaction, adaptations and strategies which will improve signal efficacy and reduce the influence of non-target audience are expected to evolve as well. A particular interesting co-evolutionary interaction of a host and an eavesdropper parasite is that between male frogs and acoustically-oriented parasitic flies, mainly frog-biting midges of the genus *Corethrella* (Corethrellidae). The interaction between females of *Corethrella* – only females have the mouth parts used to bite – and calling males of frogs is thought to date from the Lower Cretaceous, shortly after both groups arose (Borkent 2008; Borkent and Grafe 2012). Borkent (2008) argued that frog-biting midges must likely have been important in the history of frog evolution, based on the facts that both groups have been interacting for at least 190 million years, that female midges can transmit trypanosomes when obtaining a blood-meal on frog calling males, that frogs get irritated by their bites and that both groups are sympatric in most regions worldwide. Such interaction have been most well documented for frogs and midges from North America (McKeever 1977), Central America (Bernal et al. 2006) and Asia (Toma et al. 2005; Grafe et al. 2008; Borkent and Grafe 2012). Besides *Corethrella*, other genera of diptera are also known to act as parasites of frogs, some of them with species known to be attracted to frog calls, such as some species of *Uranotaenia* (Culicidae) (Borkent and Belton 2006), whereas for other taxa the specific cues used to detect their frog hosts are not well know, as is the case of the *Forcipomyia* (Ceratopogonidae) (Spinelli et al. 2002) and *Sycorax* flies (Psychodidae) (Bravo and Salazar-Valenzuela 2009).

Although the interaction between acoustically-oriented parasitic flies and male frogs is a unique topic of investigation in behavioral ecology, much of the evidence is still circumstantial (McKeever 1977; Grafe and Wanger 2007; Caldart et al. 2014) and most of the studies investigating this topic was focused in lotic and/or lentic habitats from tropical areas (Bernal et al. 2006; Grafe et al. 2008). To date, we are aware of only two circumstantial records of the interaction between parasitic flies – although not necessarily acoustically-oriented – and frog hosts for the southern Neotropics: between *Forcipomyia* flies and the frogs *Leptodactylus chaquensis* and *Pseudis paradoxa* from Argentina (Spinelli et al. 2002), and between an unknown taxa of parasitic flies and the stream frog *Crossodactylus schmidti* from southern Brazil (Caldart et al. 2014). Thus, the pattern of richness and abundance of these parasitic flies is not well known for the austral portion of the Neotropical region, neither if these flies are attracted to frog calls and which taxa can be regarded as a potential selective pressure on male calling frogs (see Borkent 2008).

Herein, we investigated the interaction between parasitic flies and male frogs of three species at an area of preserved seasonal forest from southern Brazil. We performed phonotaxis experiments in the field in order to address the following question: which taxa of parasitic flies are acoustically-oriented toward the calls of anuran species typical of forested areas in southern Brazil? To do so, we broadcasted the calls of three species which have a relatively wide distribution in forests of the austral Neotropics: *Crossodactylus schmidti* (Hylodidae; Caldart et al. 2013), *Physalaemus* cf. *gracilis* (Leptodactylidae; Kwet et al. 2010) and *Scinax perereca* (Hylidae; Kwet et al. 2010; Frost 2015). Furthermore, we also included a treatment with a white noise stimulus to compare the capture patterns between the taxa of parasitic flies. This is the first study to address the interaction between parasitic flies and male frogs in the austral Neotropics using different phonotaxis treatments.

2. Material and Methods

The study was conducted between October 2013 and February 2014 (austral spring and summer) in streams and ponds within the Turvo State Park (27°14'34.08"S, 53°57'13.74"W, 376 m.a.s.l.), located in the municipality of Derrubadas, northwest region of the state of Rio Grande do Sul, Brazil. The park covers an area of 17,491.4 ha (SEMA 2005) adjacent to the border of the Uruguay River and near the Argentinean forests of the Moconá Provincial Park and the Yabotí International Biosphere Reserve, and is one of the last large remnants of mesophytic semideciduous forest (*sensu* Oliveira-Filho et al. 2006) in southern Brazil. Local climate is characterized as subtropical sub-humid with dry summer, with an annual rainfall of 1,787 mm, a mean annual temperature of 18.8 ºC and a mean temperature of the coldest month of 13.3 °C (ST SB v climate type, *sensu* Maluf 2000).

We performed phonotaxis experiments by broadcasting frog calls from three pairs of suction traps (modified from Mckeever and Hartberg 1980), which consisted in a PVC pipe with a suction device in their midst, and a funnel at the top with an attached plastic container filled with 80% alcohol for collecting the sucked flies. At the base of the trap a small battery was placed to power the suction device, and attached near to the opening of the funnel we placed a loudspeaker (Sony ICD-PX333; overall frequency response: 75 – 20,000 Hz) to broadcast the frog calls (Appendix B). The pairs of traps were disposed in ponds and streams during trapping sessions of 45 minutes in the diurnal period (11:00 to 17:00) and at the dusk/nocturnal period (18:00 to 23:00). Each pair consisted in an acoustic trap broadcasting frog calls and an adjacent silent control trap, placed approximately 5 m distant from the acoustic trap. Pairs of traps were placed approximately 20 m apart from each other to avoid acoustic interference. Between two followed sessions we switched the stimulus between pairs of traps (i.e. the acoustic trap became the silent trap, and vice-versa) to control for the possibility of the equipment attracting flies. We performed a total of 139 trapping sessions, totaling ~105 hours; we did from four to seven diurnal trapping sessions per day, whilst dusk/nocturnal trapping session varied from 4 to 10 per day.

We played calls of three anuran species commonly found in the study area: one typical of streams, the frog *Crossodactylus schmidti* (Hylodidae) and two typical of ponds, the frog *Physalaemus* cf. *gracilis* (Leptodactylidae) and the tree-frog *Scinax perereca* (Hylidae). Measurements of call parameters were obtained with SoundRuler software v. 0.9.6.0 (http://soundruler.sourceforge.net; (Bee 2004; Gridi-Papp 2004) at a sampling frequency of 44100 Hz and a resolution of 16 bits, from calls of six males of *C. schmidti* and of *S.*

perereca, and five males of *P.* cf. *gracilis*. Then, for each anuran species we created a playback loop based on their call mean parameters using the software Adobe Audition. The playback loop of *C. schmidti* calls (Appendix C) consisted in a long trill of 15 s call with 53 notes, emitted in a call rate of two calls per minute, with 15 s of silent interval between calls and a call mean frequency of 3350 Hz, equalized at an amplitude of 70 dB in front of the loudspeaker prior to each session with a decibelimeter (Impac®, model IP-120C). Differently from *C. schmidti*, whose nearby males call with little acoustic overlap (Caldart et al. 2011), males of the frog *P.* cf. *gracilis* and the tree-frog *S. perereca* call in dense choruses (Iop et al. 2012). The playback loop of *P.* cf. *gracilis* calls (Appendix D) consisted in a 90 s chorus composed by whine calls of approximately 5 s each, emitted in a call rate of 10 calls per minute, with a silent interval of 41 s between choruses and a call mean frequency of 2230 Hz, equalized at an amplitude of 69 dB. The loop of *S. perereca* calls (Appendix E), in turn, consisted in a 23 s chorus composed of short calls of 0.5 s, emitted in a call rate of 114 calls per minute, with a silent interval of 29 s between choruses and a call mean frequency of 3129 Hz, equalized at an amplitude of 60 dB. Furthermore, we also broadcasted a loop of a white noise of 5 s duration with silent interval of 5 s between noise periods, composed by frequencies ranging from 0–10000 Hz and equalized at 66 dB (i.e., comprising the frequency range and the amplitude of the three anuran calls; Appendix F).

To identify the flies to the genera level, we consulted taxonomic studies which present morphological diagnostic characters (Appendix G-I) of the genera that we could potentially capture (i.e. *Corethrella*, *Forcipomyia*, *Uranotaenia* and *Sycorax*): Thielmann and Hunter (2007), Marino and Spinelli (2008), Borkent (2008) Bravo and Salazar-Valenzuela (2009), Santos and Bravo (2009), Borkent and Grafe (2012) and Brown (2009). For statistical analysis, we compared the number of female flies captured in acoustic and control traps for all anuran species with Mann-Whitney tests, and for each anuran species and the white noise separately, we compared the frequency of captured female flies between acoustic and silent traps with Chi-square tests, due to high values of standard deviation in these data. Analyses were made in the Past software version 2.17c (Hammer et al. 2001) with a significance level of $P < 0.05$.

3. Results

We did a total of 139 trapping sessions, totaling approximately 105 hours, from which 59 sessions (43% of total) collect at least one of the target taxa of diptera. Most of the collected specimens were midges of the genus *Corethrella* (n = 188), followed by specimens of *Forcipomyia* (n = 80) and *Uranotaenia* (n = 16). *Corethrella* flies were captured only at dusk and at night (33 from 99 dusk/nocturnal sessions; 33%), as well as *Uranotaenia* (10 from 99 dusk/nocturnal sessions; 10%). Differently, *Forcipomiya* flies were captured both during the day and at night (30 from 137 diurnal/nocturnal sessions; 22%). Flies of the genera *Sycorax* (Psychodidae) were not collected.

Differences in number of specimens of *Corethrella* between acoustic and silent traps were significant with treatments analyzed altogether (U = 228.5, df = 23, P = 0.023), and differences in frequency were also highly significant with frog species analyzed separately (Table 1, Figure 1-2). Moreover, differences in the proportion of *Corethrella* between acoustic and silent traps within the same type of habitat were highly significant as well (Streams: χ^2 = 55.74; df = 12; P <0.001; Ponds: χ^2 = 42.28; df = 19; P <0.001). On the other hand, the number of captured specimens of *Forcipomyia* and *Uranotaenia* did not differ between acoustic and silent traps, neither with treatments of all frog species together (*Forcipomyia*: U = 309.5, df = 24, P = 0.954; *Uranotaenia*: U = 26.5, df = 7, P = 0.564) nor their proportions differed with frog species separately (Table 1). The comparison between traps broadcasting a white noise and the adjacent silent control traps, in turn, showed that the frequency of flies did not differ for any of the collected taxa (*Forcipomyia*: χ^2 = 2.93; df = 4; P = 0.569; *Uranotaenia*: χ^2 = 2.0; df = 1; P = 0.157; *Corethrella*: χ^2 = 5.0; df = 4; P = 0.287), confirming the pattern found in the previous tests, i.e., that *Corethrella* flies were indeed attracted to the frog calls, whereas *Forcipomyia* and *Uranotaenia* flies were captured in traps by chance.

We found five clearly distinct morphotypes of *Corethrella* based on overall morphological characteristics such as size class, coloration of body and legs, shape and pattern of wings (Table 2, Appendix G). We collected four morphotypes of *Corethrella* at both ponds and streams, with one exclusive morphotype for each habitat. We couldn’t classify only three individuals into morphotypes due to their damaged body structures. Along with nearly all females of *Corethrella* caught during trapping sessions, we also collected many male individuals.

Table 1. Mann-Whitney and Chi square tests' results of comparisons between parasitic flies collected in acoustic traps broadcasting frog calls or a white noise and adjacent control silent traps. Values in bold refer to statistically significant differences, and "N/A" refer to a result not available due to a too low capture rate.

Treatments	Taxa of parasitic flies		
	Corethrella spp.	*Forcipomyia* spp.	*Uranotaenia* spp.
Crossodactylus schmidti	$\chi^2 = 50.5$, df = 7 **$P < 0.001$**	$\chi^2 = 23.7$, df = 18 $P = 0.126$	$\chi^2 = 2.4$, df = 1 $P = 0.121$
Physalaemus cf. *gracilis*	$\chi^2 = 22.0$, df = 9 **$P = 0.009$**	$\chi^2 = 0.90$, df = 1 $P = 0.342$	$\chi^2 = 6.3$, df = 4 $P = 0.178$
Scinax perereca	$\chi^2 = 16.5$, df = 7 **$P = 0.022$**	$\chi^2 = 3.27$, df = 5 $P = 0.512$	N/A
white noise	$\chi^2 = 5.0$, df = 4 $P = 0.287$	$\chi^2 = 2.93$, df = 4 $P = 0.569$	$\chi^2 = 2.0$, df = 1 $P = 0.157$
all species	$U = 228.5$, df = 23 **$P = 0.023$**	$U = 309.5$, df = 24 $P = 0.954$	$U = 26.5$, df = 7 $P = 0.564$

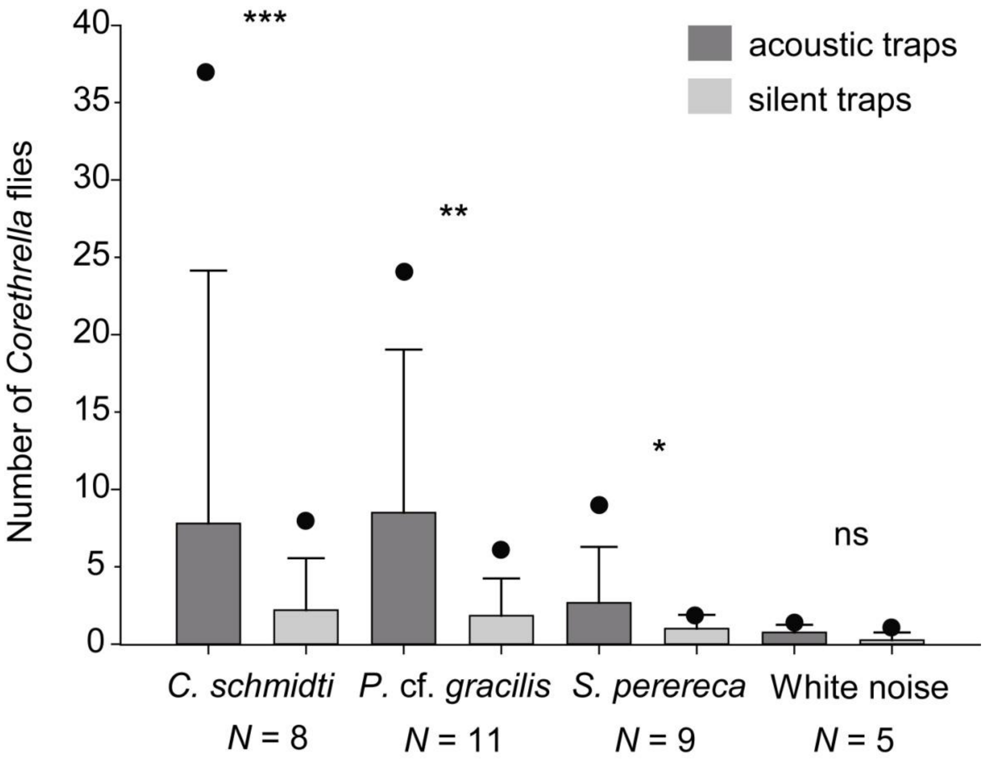

Figure 1. Number of frog-biting midges (*Corethrella*) captured in acoustic traps broadcasting advertisement calls compared with control silent traps. Boxes refer to the mean of flies captured with standard deviation above them, and the black dots refer to the maximum abundance of midges collected in a single trapping section. ***P <0.001; **P <0.01; * P <0.05, ns = not significant difference (Chi-squared tests).

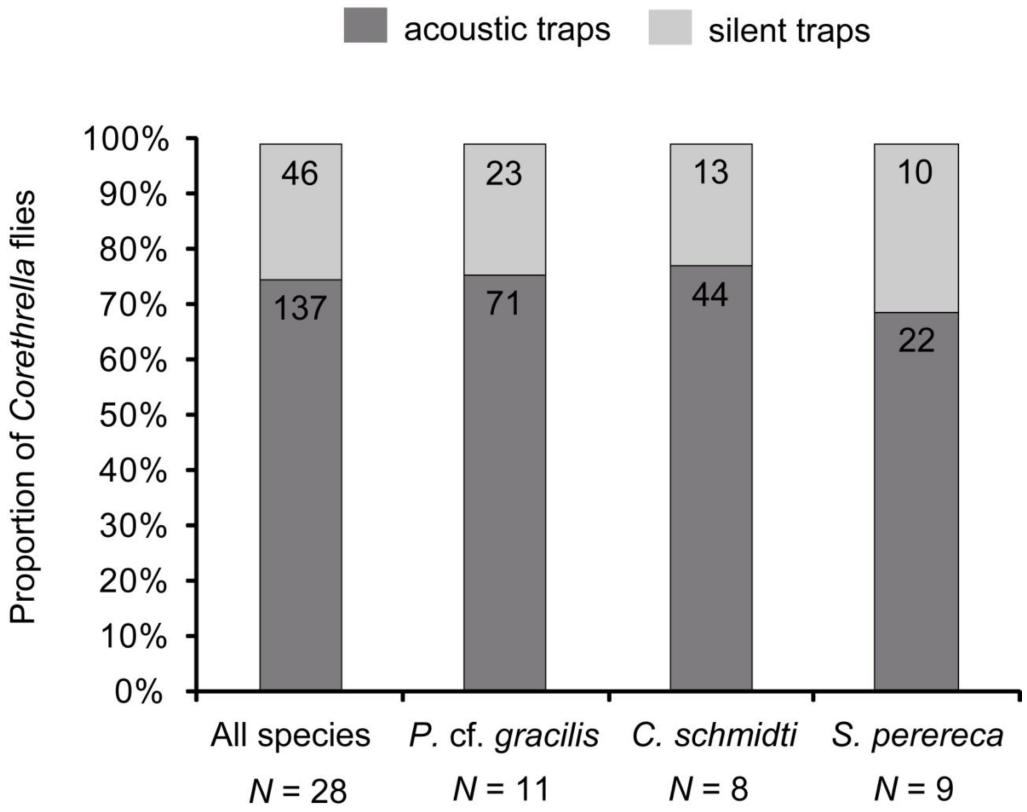

Figure 2. Proportion of frog-biting midges (*Corethrella*) captured in acoustic traps broadcasting advertisement calls compared with control silent traps for all species and for species individually. Values inside boxes refer to the total number of midges captured, and the *N* below each treatment refers to the number of sessions with captured midges.

Table 2. Description of the morphotypes of *Corethrella* captured through acoustic traps broadcasting advertisement calls of frogs, from ponds and streams in southern Brazil.

Morphotype	General description	Stream	Pond	Total
(1) "Black, smooth wings"	Small size class; dark brown torax, abdomen and legs; ligth yelowish wings	X		82
(2) "Black, patched wings"	Small size class; dark brown torax and abdomen; ligth brown legs with dark patches; ligth yellowish wings with black patches	X	X	65
(3) "Bee-like body"	Medium size class; yellowish body with dark stripes in abdomen; yellowish legs; ligth yelowish, hairy wings	X	X	35
(4) "Hairy legs"	Medium size class; brown torax and abdomen; brown patched, hairy legs; light yellowish wings with black patches	X	X	2
(5) "Mild-patched wings"	Medium size class; brown torax, dark brown abdomen; light brown pacthed legs; light yellowish wings with mild black pacthes		X	1

4. Discussion

Our study examined and described for the first time the pattern of attraction of parasitic flies to frog calls in the austral portion of the Neotropical region, showing that the calls of *Crossodactylus schmidti*, *Physalaemus* cf. *gracilis* and *Scinax perereca* are used as acoustic cues by female flies of *Corethrella*, whereas on the other hand, the calls of these frogs are not used as acoustic cues by flies of the genera *Forcipomyia* and *Uranotaenia*. Other recent studies also found that *Corethrella* flies were attracted to the acoustic cues of frog calls (Bernal et al. 2006; Grafe et al. 2008), but information on *Forcipomyia* and *Uranotaenia* is still scarce and the use of a white noise stimulus to support comparisons between taxa is not usual. In our study, the pattern of attraction of *Corethrella* flies to the treatments with frog calls – whilst flies of *Forcipomyia* and *Uranotaenia* were not attracted – is strongly supported by the fact that the proportions of these taxa did not differ between the acoustic traps broadcasting a white noise stimulus and the adjacent silent traps.

We found a pattern of much lower abundance of *Corethrella* flies at our study site, when compared to what have been registered for tropical areas. For instance, whilst in a site of Borneo Grafe et al. (2008) collected an average of 37 flies per hour of trapping, with abundance ranging from 0 to 268, we collected an average of just three flies per hour, with abundance ranging from 0 to 37 flies per trapping session. Though our study is not conclusive regarding the abundance pattern of this taxon in the austral Neotropics, the low abundance at our site is not surprising, since *Corethrella* are most diverse and abundant in lowland tropics, and because our study site (latitude 27º S) is located near to the austral limit of distribution known for this taxon in South America, located at the latitude of 30º S near Buenos Aires, Argentina (Borkent 2008). Besides, the pattern of richness and abundance of *Corethrella* may also varies depending on the frog species used as baits, as well as according to the type of habitat sampled (Borkent 2008; Grafe et al. 2008).

The low abundance of *Corethrella* flies at our study site suggests that their pressure in the calling activity of male frogs is lower as compared to that of tropical sites (see Bernal et al. 2006; Grafe et al. 2008). Furthermore, as *Corethrella* flies were collected only at dusk/night period, the potential pressure of the exploitation of acoustic signals by these flies may be higher on males of nocturnal frogs, because the period of activity of both flies and frogs overlaps. Indeed, *Corethrella* flies are thought to have evolved a strategy to feed on nocturnal frogs because most species of frogs vocalize at night and both groups are coevolving since at least from the Lower Cretaceous (Borkent 2008). Although we used calls of two typical nocturnal species, at least 15 anuran species were recorded in nocturnal calling activity in the study area during the austral spring and summer (Iop et al. 2012), which suggests that interactions between *Corethrella* and male frogs of other species are likely to occur. We also collected many male individuals of *Corethrella* in nearly all trapping sessions in whose female *Corethrella* were caught. According to Borkent (2008), males of *Corethrella* are also attracted to calling frogs to find conspecific females, and Silva and Bernal (2013) documented that the swarms of *Corethrella appendiculata* consisted predominantly of males, indicating a male-biased, lek-like mating system. Given that we already know that *Corethrella* flies are attracted to acoustic cues of frog calls at our study site, further studies broadcasting calls of different frog species simultaneously would be interesting to test the degree of host specificity in the interactions (see Grafe et al. 2008).

Considering the pattern of a predominantly diurnal calling activity of the frog *Crossodactylus schmidti* (Caldart, unpublished data), we argue that there’s only a small temporal window (at dusk) in which *Corethrella* flies are able to parasitize calling males of this species in the study area. This may also suggest that the flies attacking the calling male of *C. schmidti* in the interaction described by Caldart et al (2014) are not *Corethrella*, but instead could be any other taxa of diurnal parasitic flies which are attracted to hosts by cues other than acoustic, e.g., visual cues, carbon dioxide, water vapour or other volatile skin secretion (Borkent and Belton 2006). This finding is important as it draws attention to the need of studies including an experimental design aiming to test the attraction of parasitic flies to other types of cues from frog hosts. Regarding the habitat of the frogs, most of the morphotypes of *Corethrella* occurred at both ponds and streams, which is in agreement with the idea that mating swarms of *Corethrella* are formed near the habitats where anurans aggregate for reproduction (Borkent 2008; Silva and Bernal 2013).

Flies of the genera *Forcipomyia* and *Uranotaenia* were randomly captured in both acoustic and silent traps. Regarding *Uranotaenia* spp., we expected to collect much more individuals with frog calls, given that some studies have found such pattern in Costa Rica (Borkent and Belton 2006) and in the southern islands of Japan (Toma et al. 2005). However, this result do not preclude the existence of an interaction between *Uranotaeni*a flies and male calling frogs in southern Brazil, but instead may suggest that species of *Uranotaenia* can use cues other than acoustic cues, or may respond to call frequencies which are particularly sensitive to their antennae. The antennae of *Uranotaenia lowii*, for example, are thought to resonate at around 400-500 Hz of the fundamental harmonic of the calls of *Hyla gratiosa*, although experiments to confirm this are still lacking (Borkent and Belton 2006). For

Forcipomyia, in turn, our results indicate that these flies are not attracted by the acoustic cues of frog calls. Although these flies may attack frogs (Spinelli et al. 2002), they must use cues other than acoustic ones to find their hosts, since they are known to parasitize wings of dragonflies (Odonata) in the Neotropical region as well (Guillermo-Ferreira and Vilela 2013).

This work constitutes the first study to investigate the interaction between parasitic flies and frog hosts using different phonotaxis treatments in the austral portion of the Neotropical region. By considering different taxa of possible parasitic flies and by including a white noise treatment in our design, we demonstrated an interesting pattern regarding the attraction of these flies to the acoustic cues of frogs: we reported that flies of the genus *Corethrella* were the only taxon of parasitic flies unequivocally known to be attracted to calls of *C. schmidti*, *P.* cf. *gracilis* and *S. perereca*, a pattern reinforced by the lack of differences between the white noise stimulus and the adjacent silent traps. However, the low abundance of *Corethrella* in the austral neotropics suggests a lower pressure of these flies in male frogs, when compared to what have been documented for tropical areas (Bernal et al. 2006; Borkent 2008; Grafe et al. 2008). Given that the interaction between parasitic flies and frog hosts are a unique example of coevolution (Ferguson and Smith 2012), studies on this topic on the austral Neotropics must be encouraged to a better understanding of the cues used by the flies to locate their hosts, the degree of host specificity in the interactions and the evolution of adaptations in both hosts and parasites.

Acknowledgements

We are grateful to Art Borkent and Thomas Grafe for kindly providing information about the interaction between parasitic flies and frogs and identification of the diptera taxa, to to Camila Both, Cynthia Prado, Selvino Neckel de Oliveira and Sandro Santos for valuable suggestions that greatly improved this study, and to the staff of the ‘Laboratório de Herpetologia – UFSM’ for their assistance in field activities. V.M.C. and S.I. thank CAPES for the doctoral fellowships and S.Z.C. is grateful to CNPq for the award of a research fellowship (No. 304929/2012-3). Activities in Turvo State Park were performed in accordance with the access licenses issued by SEMA-RS (#23/2011) and ICMBio-IBAMA (#29505-4).

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CONCLUSÕES

— A distribuição geográfica de *Crossodactylus schmidti* está associada ao Núcleo Misiones das Florestas Tropicais Sazonalmente Secas e seus enclaves de Floresta Estacional nos estados Brasileiros do Paraná, Santa Catarina e Rio Grande do Sul, no sul do Paraguai e no nordeste da Argentina. Na classificação de Ecorregiões, a distribuição da espécie está inteiramente inserida na Ecorregião da Floresta do Alto Paraná e em áreas adjacentes de transição com a Ecorregião da Floresta de Araucária.

— No estado do Rio Grande do Sul, os registros de *C. schmidti* sugerem que algumas populações encontram-se ameaçadas pela redução das florestas, a expansão de culturas agrícolas e de usinas hidrelétricas, a exemplo do que ocorre também no oeste de Santa Catarina. Estudos de dinâmica populacional, localização de novas populações e medidas de conservação dos ambientes lóticos e das florestas adjacentes são ações necessárias para a conservação da espécie.

— A comunicação em *C. schmidti* envolve um amplo repertório de sinais acústicos, visuais e multimodais. O repertório de sinais visuais inclui nove tipos de sinais. A evolução de um repertório de sinais tão diverso é atribuída a *1) fatores históricos*, como a atividade diurna e a presença histórica em ambientes acusticamente complexos, *2) a fatores comportamentais*, como o uso de sítios para emissão de sinais que favorecem a emissão dos sinais (i.e. maior razão sinal-ruído) e as frequentes e prolongadas interações agonísticas entre machos e entre adultos e juvenis, e *3) a fatores ecológic*os, como a potencial pressão seletiva imposta por mosquitos parasitas de anuros.

— A ocorrência de comportamentos derivados similares a sinais visuais, observados em contextos não sociais diversos, sugere que o processo de ritualização dessas posturas e/ou movimentos no passado deve ter favorecido a evolução de pelo menos parte do repertório visual de *C. schmidti*, dado que comportamentos derivados são considerados os precursores de sinais na comunicação animal, e o processo de ritualização constitui o principal meio pelo qual os sinais animais evoluem.

— *Crossodactylus schmidti* possui atividade de canto contínua no sul do Brasil, que é influenciada por fatores ambientais de formas diferentes. O efeito da chuva acumulada e da temperatura do ar é importante para regular a variação mensal na atividade, enquanto que a intensidade luminosa e a temperatura do ar exercem efeito na variação da atividade ao longo do dia.

— Na escala mensal, as variáveis que explicam a variação na atividade de canto devem ter relação com *1)* o modo reprodutivo *C. schmidti* (modo 3: ovos depositados em câmaras subaquáticas de riachos), pois fortes chuvas afetam a dinâmica espacial dos riachos e a disponibilidade de sítios de canto e oviposição, e com *2)* o desempenho individual de canto, que em anuros é influenciado pela temperatura do ar.

— Na escala diária, o efeito da temperatura do ar sobre a atividade de canto das primeiras horas do dia (05:00 e 06:00) deve guardar relação com a possibilidade de haver uma maior eficácia na transmissão do sinal acústico nesse período, dado que no início da manhã as condições atmosféricas são mais estáveis em relação ao restante do dia. Entretanto, o efeito de fatores intrínsecos, como o ritmo circadiano, também deve ter relação com o pronunciado e consistente pico de atividade de canto do início da manhã.

— O efeito da intensidade luminosa ao longo do dia (07:00 às 19:00) , por sua vez, tem relação com o fato de que a transmissão de sinais visuais isolados, ou como parte de um display audiovisual, deve ser favorecida quando uma maior intensidade luminosa atinge aos sítios de vocalização dos machos. Como muitos sinais visuais são frequentemente emitidos associados a sinais acústicos, sítios de vocalização onde há maior intensidade luminosa devem proporcionar ao emissor um aumento na razão sinal-ruído do componente visual do display.

— Os padrões temporais de atividade de canto observados devem exercer influência sobre o sistema de acasalamento da espécie, que ocorre primariamente através da escolha de machos com base na qualidade dos recursos dominados por este, i.e., seleção sexual com base na qualidade dos sítios de canto e/ou sítios de oviposição e, secundariamente, na seleção de machos com base na sua performance de canto, i.e., seleção a partir da variação nos traços acústicos individuais.

— *Crossodactylus schmidti* apresenta adaptações históricas (*long-term adaptations*) para a comunicação, relacionadas à presença histórica num ambiente acusticamente complexo, bem como plasticidade (*short-term adaptations*) na emissão de alguns traços acústicos relacionada às mudanças súbitas ou temporárias no ruído ambiente. Ambas as formas de adaptações permitem ao emissor aumentar a razão sinal-ruído.

— O canto longo e trinado, composto por várias notas, é interpretado como uma adaptação acústica em resposta ao fato de que algumas notas do canto apresentam razão sinal-ruído negativa frente ao mascaramento do ruído do riacho. Da mesma forma, a estrutura harmônica do canto e o uso de sinais visuais isolados ou inseridos num display audiovisual devem ter evoluído devido ao efeito de mascaramento do ruído ambiente sobre a transmissão de sinal acústico (*long-term adaptations*).

— As mudanças plásticas observadas em traços acústicos, como a modulação da frequência dominante, a variação na localização da frequência dominante entre harmônicos, e o aumento na frequência das notas ao longo do canto, por sua vez, constituem estratégias comportamentais para lidar com mudanças temporárias e ou súbitas no ruído ambiente (*short-term adaptations*).

— O padrão de atividade reprodutiva de *Crossodactylus schmidti* no sul do Brasil é do tipo contínuo, com a presença de indivíduos sexualmente maduros de ambos os sexos ao longo do ano todo. Embora a proporção de indivíduos maduros varie mais para as fêmeas do que para os machos, não há variação sazonal significativa no comprimento rostro-cloacal, massa corpórea ou índice gonadossomático de machos e fêmeas, o que reforça o padrão contínuo da atividade reprodutiva. Além disso, machos em atividade de canto e girinos livres também ocorrem o ano todo.

— Há dimorfismo sexual em duas características morfológicas: no comprimento rostro-cloacal (CRC) e na largura da boca (LB). Fêmeas foram significativamente maiores no CRC do que os machos e, com o efeito do CRC controlado, fêmeas possuem LB significativamente maior do que machos. O CRC maior em fêmeas guarda relação com ganho reprodutivo associado a um maior tamanho corpóreo, dado que a massa corpórea teve um forte efeito positivo na massa dos ovários (CRC e massa corpórea são traços altamente correlacionados). Por outro lado, uma maior LB em fêmeas deve estar associada com diferenças sexuais no nicho trófico.

— A evidência advinda das análises histológicas das gônadas demonstra que em alguns meses a proporção de fêmeas maduras cai pela metade, enquanto que a proporção de machos maduros continua alta. Esse resultado dá suporte ao argumento de que há uma forte competição intrasexual em *C. schmidti*, particularmente para os machos, e ajuda a explicar por que machos em atividade de canto e em intensos combates agonísticos são observados ao longo de todo o ano.

— Tomados em conjunto, os resultados sobre a atividade de canto e sobre o padrão reprodutivo sugerem que o sistema de acasalamento de *C. schmidti* é do tipo *"poliginia por defesa de recursos"*. Assim, indivíduos maiores devem ter maior vantagem para a reprodução, dado que machos maiores devem sair beneficiados ao estabelecer e defender recursos importantes, como territórios que possuam sítios de canto e locais propícios para a oviposição, enquanto que fêmeas maiores devem ter mais vantagem em relação a fêmeas menores porque podem potencialmente deixar uma desova maior ou mais numerosa.

— A captura de mosquitos do gênero *Corethrella* foi significativamente maior em armadilhas acústicas do que em armadilhas silenciosas, com as espécies de anuros analisadas em conjunto ou separadamente. Em contrapartida, a captura de mosquitos dos gêneros *Forcipomyia* e *Uranotaenia* não diferiu entre armadilhas acústicas e silenciosas.

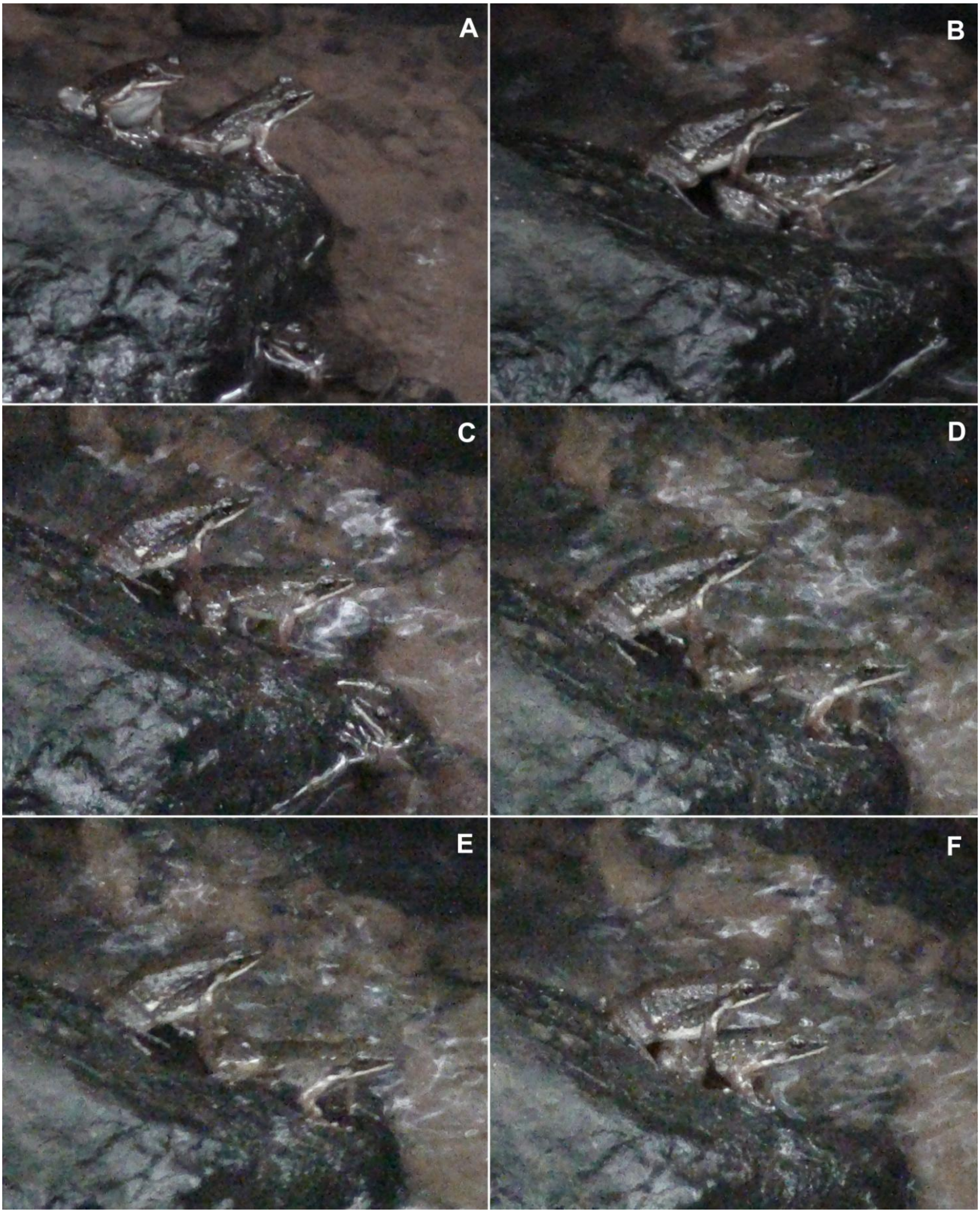
— Reforçando este padrão, a comparação entre as armadilhas emitindo um ruído branco e as armadilhas de controle silenciosas demonstrou que as proporções de mosquitos não diferiu para nenhum dos táxons coletados, o que sugere fortemente que os mosquitos do gênero *Corethrella* são de fato atraídos por cantos de anuros, enquanto os dos gêneros *Forcipomyia* e *Uranotaenia* não são atraídos pelo estímulo acústico de cantos de anuros.

— Assim, dentre os táxons de mosquitos avaliados, mosquitos do gênero *Corethrella* representam o único táxon confirmadamente atraído por cantos de *Crossodactylus schmidti*, *Physalaemus* cf. *gracilis* e *Scinax perereca* para a região sul do Brasil, embora a abundância de mosquitos do gênero *Corethrella* na região neotropical austral é muito menor se comparada ao padrão que tem sido registrado em áreas tropicais.

— Considerando os resultados apresentados nesta tese e os dados de história natural já existentes, conclui-se que *C. schmidti* representa de fato uma espécie importante do ponto de vista de estudos sobre evolução da comunicação e reprodução em anuros diurnos de riacho, de modo que deve ser utilizada como organismo modelo em estudos que visem testar hipóteses sobre esses tópicos.

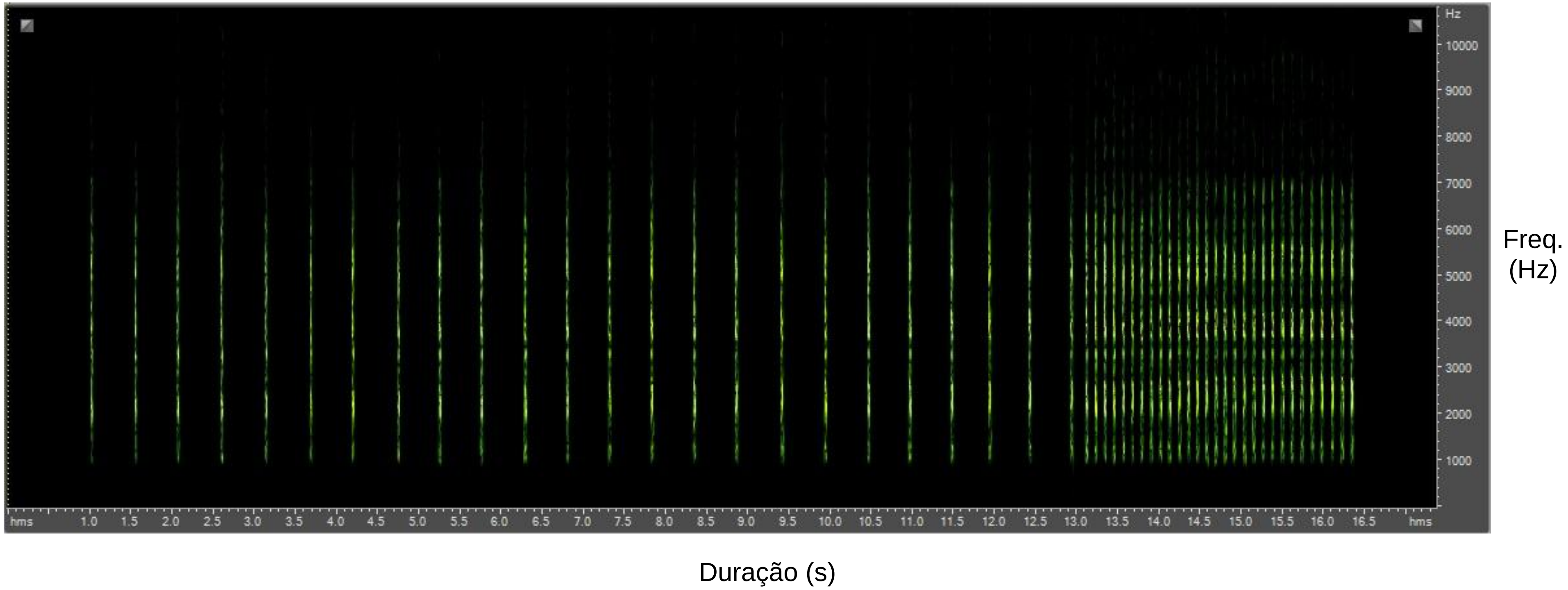
APÊNDICES

APÊNDICE A: Sequência de comportamentos entre um casal de *C. schmidti* (A–F) e um macho invasor (A e C, canto inferior direito). O macho vocaliza a partir do seu sítio de canto em direção ao macho invasor (C), e a fêmea posicionada atrás do macho vocalizante interage tactilmente com este através de uma postura que lembra um amplexo inguinal (F).

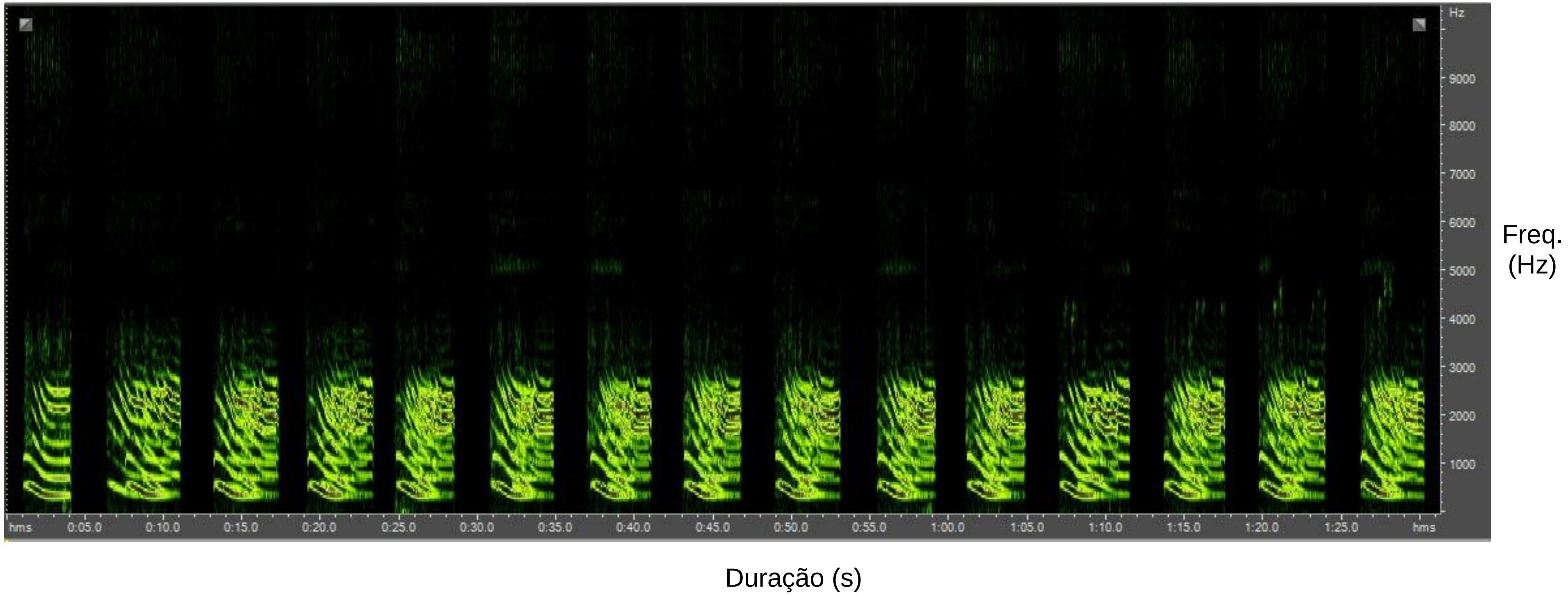


APÊNDICE B: Armadilhas de sucção utilizadas para coleta de potenciais mosquitos parasitas de anuros em riachos (A e D) e poças (B e C) em uma área de floresta estacional semidecídua no sul do Brasil. Cada armadilha (E) consistia em um tubo de PVC com um dispositivo de sucção em seu meio, e um funil no topo com um recipiente de plástico contendo álcool 80% para a coleta dos insetos; próximo à abertura do funil coletor um gravador com alto-falante reproduzia os cantos dos anuros e na base da armadilha uma bateria de 12 V alimentava o dispositivo de sucção.

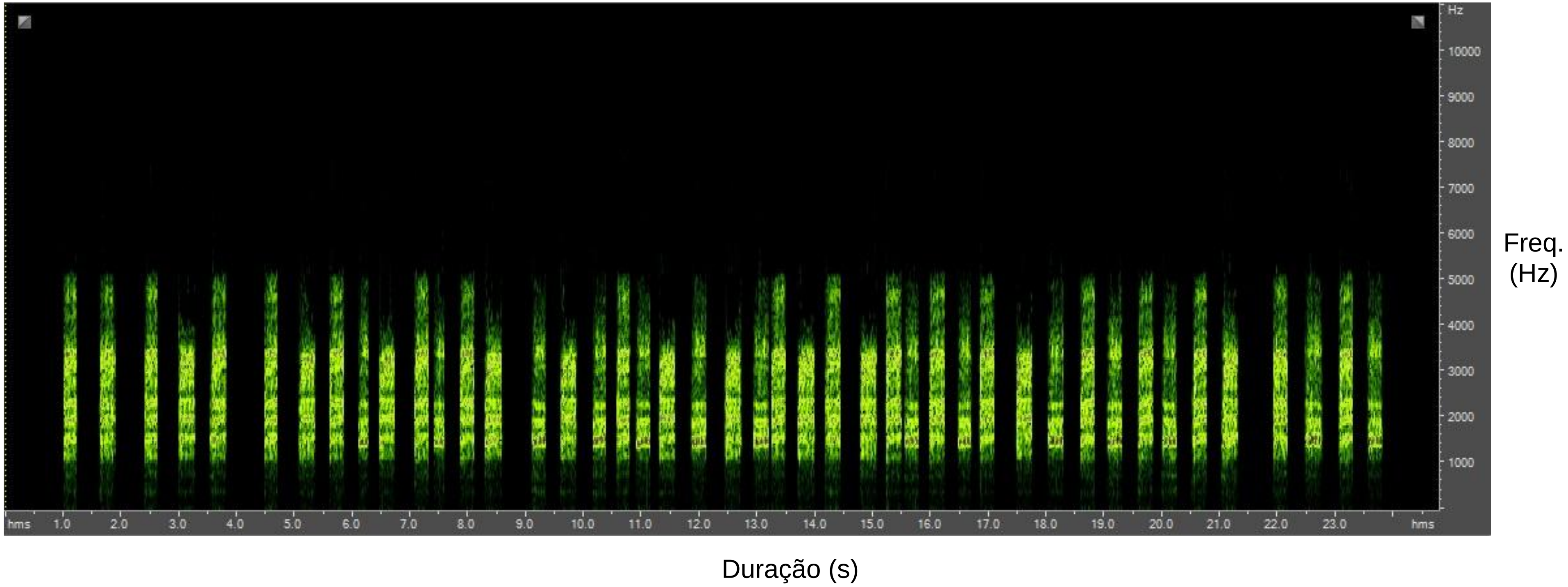
APÊNDICE C. Canto de *Crossodactylus schmidti* reproduzido durante as sessões de captura de potencias mosquitos parasitas de anuros.



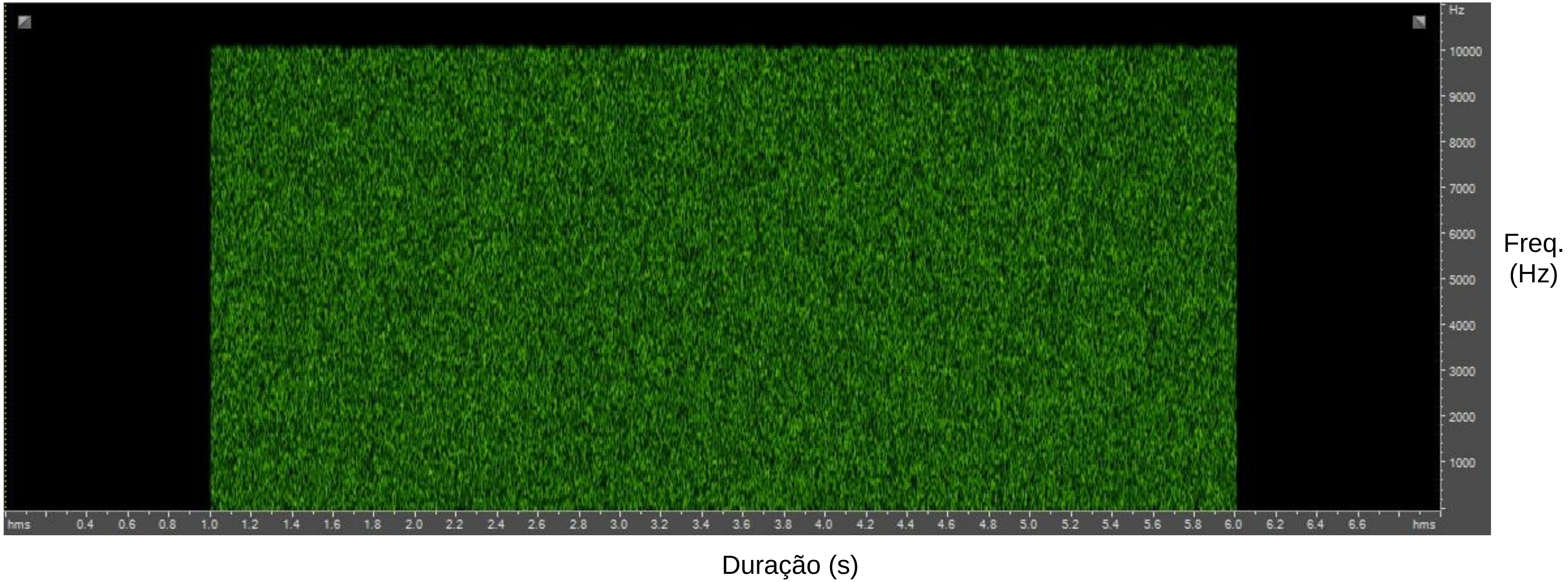
APÊNDICE D. Coro de cantos de *Physalaemus* cf. *gracilis* reproduzido durante as sessões de captura de potencias mosquitos parasitas de anuros.



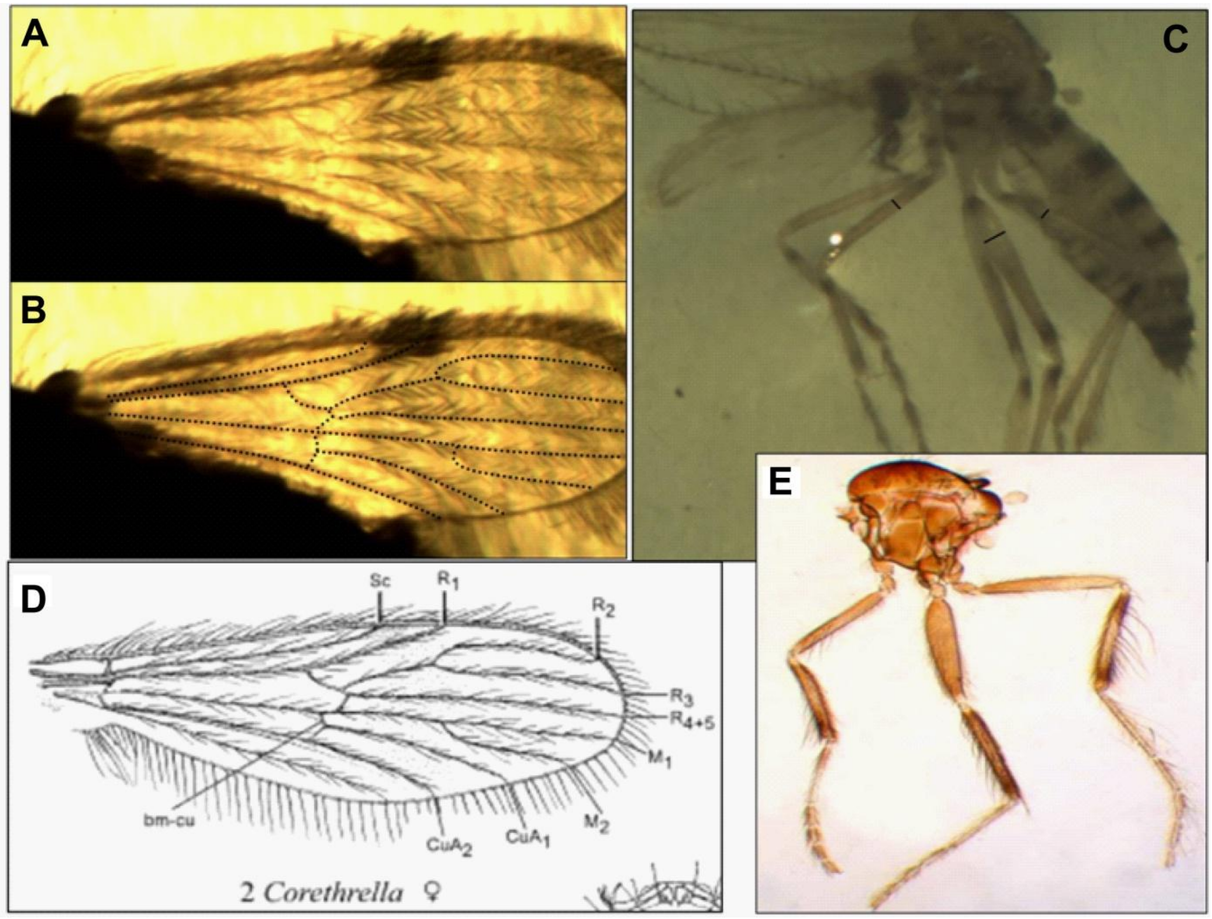
APÊNDICE E. Coro de cantos de *Scinax perereca* reproduzido durante as sessões de captura de potencias mosquitos parasitas de anuros.



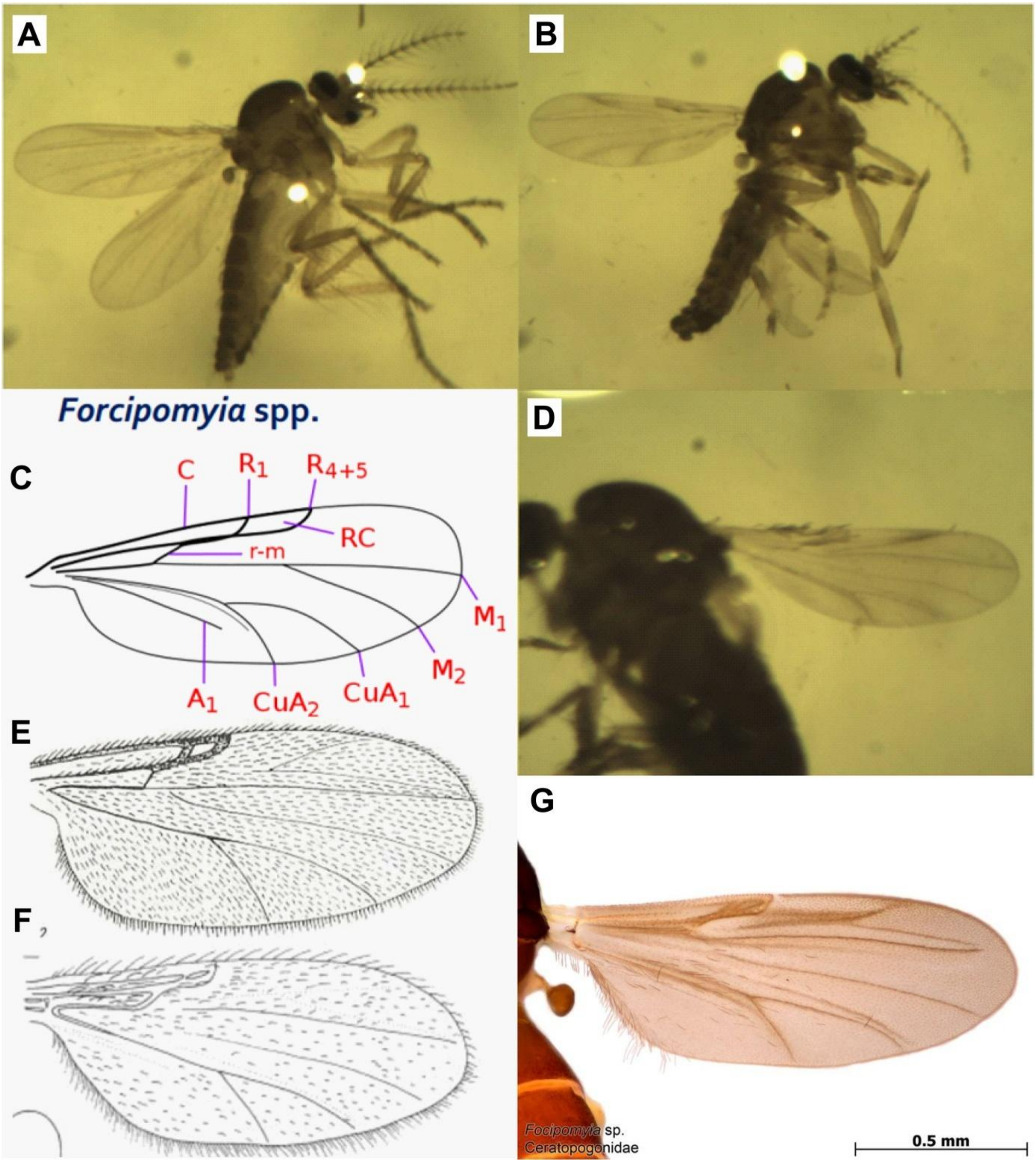
APÊNDICE F. Ruído branco reproduzido durante as sessões de captura de potencias mosquitos parasitas de anuros.



APÊNDICE G. Padrão geral da asa (A, B e D) e do corpo (C e E) de mosquitos fêmeas do gênero *Corethrella* (Corethrellidae). D) Esquema da asa típica do gênero *Corethrella* obtido de “Manual of Central American Diptera” (Brown et al., 2009); E) Tórax e pernas (note o fêmur do 2º par de pernas mais largo que os demais) de um *Corethrella* sp. obtido de “Frog-biting Midges of the World” (Borkent, 2008).



APÊNDICE H. Padrão geral do corpo (A – B) e da asa (C – G) de mosquitos fêmeas do gênero *Forcipomyia* (Ceratopogonidae). E – F) Esquema da asa típica do gênero *Forcipomyia* obtido de “Biting Midges of the *Forcipomyia* (*Forcipomyia*) *argenteola* group in southern South America, with description of a new species and a key to the Neotropical species (Diptera: Ceratopogonidae)” (Marino & Spinelli, 2008).



APÊNDICE I. Padrão geral do corpo (A, B, C e H), da asa (D – E) e da probóscide (F – G) de mosquitos fêmeas do gênero *Uranotaenia* (Culicidae). D) Esquema da asa típica do gênero *Uranotaenia*, obtido de “The mosquitoes (Diptera: Culicidae) of Seychelles: taxonomy, ecology, vectorial importance and identification keys” (Le Goff et al., 2012); F) Probóscide típica de um *Uranotaenia* sp., obtido de “Photographic guide to common mosquitoes of Florida” (Cutwa & O’meara).