Universidade Federal do Rio Grande - FURG
Instituto de Ciências Biológicas
Pós-graduação em Biologia de Ambientes
Aquáticos Continentais

Biologia de *Helicops infrataeniatus* Jan, 1883 (Serpentes, Dipsadidae) na planície costeira do extremo sul do Brasil

Ruth Anastasia Regnet

Orientador Prof. Dr. Daniel Loebmann

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Dissertação apresentada ao Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais como requisito parcial para a obtenção do título de Mestre em Biologia de Ambientes Aquáticos Continentais.

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Em referência a *Helicops infrataeniatus*: "*Parem cerca de 25 filhotes de cada vez e deve haver muitos predadores dessas serpentes, havendo canibalismo entre os irmãos o que é bastante providencial porque senão os rios ficariam completamente abarrotados dessas serpentes, pois elas são abundantes*."

Lema. T. de 2002.

AGRADECIMENTOS

Esta dissertação de mestrado eu considero minha maior contribuição à ciência até então. Todo o conjunto desta presente obra foi construído com muito amor pelo meu ofício de bióloga. Naturalmente é de suma importância exprimir os meus sinceros agradecimentos a algumas das pessoas que foram singulares neste ciclo que se perfaz.

Assim, início esta sessão agradecendo a meu amigo Marcos Marques, que ao final da minha graduação me apresentou o laboratório no qual estagiava, o Laboratório de Vertebrados da FURG. Isto de fato acrescentou e mudou em muito minha vida, pois o Laboratório veio a tornar-se minha segunda casa.

Agradeço aos emocionantes momentos, nos primórdios de meus conhecimentos herpetológicos, com Victor Teixeira e Fernando Quintela. Ao Victor pela presença no dramático momento do encontro com a primeira serpente em um *Pitfall* e a descoberta de como um anuro fabrica seu ninho de espuma. Ao Quintela por todos seus conhecimentos herpetológicos compartilhados, pelo primeiro jacaré capturado e por todos os devaneios em campo.

Agradeço também imensamente ao meu grande amigo Daniel Loebmann, pelo convite para participar das atividades do seu laboratório. E aos que se perguntam neste momento: mas ele não é seu orientador de mestrado? Respondo com muito orgulho que sim! Mas, além do que simplesmente ser o orientador de mestrado, é um grande amigo e orientador para a vida. Aos leitores, digo-lhes que foram muitos debates regados à café, vinho, cerveja, churrasco e porque não dizer deste projeto nascido com apoio de whisky com mel. Campos de mãos e pés gelados. Horas de aulas práticas de inglês, de buscas de bibliografias, sinônimos, construção de manuscritos e trocas de conhecimento. Como também todo o apoio nas mais diversas situações que a vida nos apresenta, talvez lhes faça compreender um pouco desta minha eterna gratidão.

Agradeço também a colega de laboratório Melise Silveira pela oportunidade de participar de seus campos, rendendo bons ensinamentos práticos de rádio-telemetria e biologia de quelônios de água doce.

Aos demais colegas de laboratório, Andressa Gomide, Claudia de Paula, Felipe Caseiro, Franck Silveira, Laíz Crizel, Omar Entiauspe-Neto e Sônia Huckembeck sou grata pelo coleguismo e pelos bons momentos juntos.

Ao querido Guilherme Sansão agradeço por sempre acreditar em meu potencial e me incentivar. Por independente do que for, eu sempre estar em seu coração, mesmo com toda sua dificuldade em compreender minha necessidade de ausência para elaboração da dissertação. Por complicar e descomplicar tanto a vida e especialmente por ser um grande companheiro para todos os momentos.

Aos meus irmãos, por serem os melhores de todos e a Dona Silvia, por ser essa minha mãe muito doida. Obrigada por terem orgulho de mim.

A Arthur Gerard, Daison Conceição, Renan Machado e Samuel Oliveira por serem amigos que sempre estiveram presentes durante este período.

Agradeço aos professores e funcionários do Programa de Pós Graduação em Biologia de Ambientes Aquáticos Continentais da FURG, pelos ensinamentos e auxílios.

Agradeço aos professores Dr. Leandro Bugoni e Dr. Luís Giasson, por aceitarem compor minha banca examinadora da dissertação de mestrado; pelas sugestões e análises, às quais na medida do possível atendi com muito carinho na versão definitiva da dissertação.

Agradeço ao Dr. Jörn Köhler, editor chefe do periódico Salamandra - German Journal of Herpetology, por todo seu tempo disponibilizado com o manuscrito do primeiro capítulo desta dissertação. Principalmente pelo *feedback* positivo por meio das palavras: '*Thank you very much. For me it is absolutely fine now*'. Ficamos realmente felizes por isto.

Ao Prof. Dr. Wolfgang Böhme, do Zoologische Forschungsmuseum A. Koenig, Alemanha, pela disposição e interesse em ajudar com o caso do terceiro capítulo desta dissertação.

E por fim agradeço a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Capes, pela concessão da bolsa de estudos, que foi fundamental para permitir uma dedicação exclusiva a esta pesquisa.

RESUMO

A presente dissertação possui três capítulos e é resultado de uma incomum aglomeração de milhares de répteis e anfíbios na Praia do Laranjal, município de Pelotas, Rio Grande do Sul, em decorrência de um evento de inundação durante o El Niño de 2015. O primeiro capítulo descreve e quantifica esse evento de agregação, onde milhares de indivíduos foram carreados para um trecho de 1000 m de praia. Nesse capítulo é discutido o efeito do El Niño em um contexto regional e sua influência sobre a fauna. Também, é discutido que eventos de inundação transportam um contigente de fauna através de balsas de vegetação que, em determinados cenários podem ser vias de colonização de novos ambientes. Os mais de 700 indivíduos de *Helicops infrataeniatus* coletados resultaram no segundo capítulo, onde foi determinada a estrutura da população, a proporção e dimorfismo sexual, relações peso-comprimento de machos e fêmeas e a descrição da variação de colorido ventral. Neste capítulo são apresentados dados sobre os parâmetros de população, os quais representam o primeiro banco de dados para a espécie, gerando uma base sólida para comparações e geração de modelos de estudo para outros répteis Squamata. No terceiro capítulo descrevemos o primeiro caso de intersexualidade para a tribo Hydropsini, que foi descoberto após o exame minucioso da amostra estudada no segundo capítulo. Assim, exploramos um caso raro de uma fêmea de *H. infrataeniatus* que apresenta características reprodutivas femininas e masculinas, apresentando um órgão semelhante ao hemipênis da mesma espécie. Nós trazemos informações morfológicas detalhadas dos genitais, contribuindo com o conhecimento do grupo e permitindo um maior conhecimento acerca da diferenciação dos sexos de répteis. Anormalidades encontradas nas estruturas reprodutivas sugerem que este indivíduo intersexual pode não ser funcional do ponto de vista reprodutivo, diferindo de outros casos descritos na literatura.

Palavras-chave: Cobra-d’água, dimorfismo sexual, Hydropsini, relação peso-comprimento, Squamata.

ABSTRACT

This dissertation has three chapters and it is the result of an unusual agglomeration of thousands of reptiles and amphibians at Praia do Laranjal, municipality of Pelotas, Rio Grande do Sul, after a flooding event occurred during the 2015 El Niño. The first chapter describes and quantifies this aggregation event, where more than 2000 individuals were transported to a stretch of 1000 m of beach. In this chapter an effect of the El Niño in a regional context and its influence on fauna are discussed. Also, it brings to light that flooding events carry a contingent of fauna through rafts of vegetation that in certain scenarios may be viable to colonize new environments. The more than 700 individuals of *Helicops infrataeniatus* collected resulted in the second chapter, which determined the population structure, sex ratio and dimorphism, length-weight relationships of males and females, and description of ventral color variation. In this chapter data on population parameters are presented, which represent the first database for the species, generating a solid basis for comparisons and generation of study models for other Squamata reptiles. In the third chapter we describe the first case of intersexuality for the Hydropsini tribe, which was discovered after a thorough examination of the sample studied in the second chapter. Thus, we explored a rare case of a female of *H. infrataeniatus* that presented female and male reproductive characteristics, presenting an organ similar to the hemipenis of the same species. We bring in detailed morphological information of the genitals, contributing with the knowledge of the group and allowing greater accuracy on the sex differentiation of reptiles. Abnormalities found in reproductive structures suggest that this intersexual individual may not be functional from the reproductive point of view, differing from other cases described in the literature.

Key-words: Hydropsini, length-weight relationships, sexual dimorphism, Squamata, water snake.

APRESENTAÇÃO

O estudo da biologia das populações de répteis squamados se mostra um grande desafio, pois o hábito criptozóico das espécies e sua relativa baixa densidade na natureza dificulta a obtenção de uma amostra ideal para interpretação dos resultados. Em anos de El Niño, o extremo sul do Brasil tem um volume esperado de chuva acima das médias históricas. Isso tem promovido ciclos de inundação nas margens da região estuarina da Lagoa dos Patos. Embora não reportado até então, as inundações causam um impacto direto nas populações de anfíbios e répteis que habitam áreas adjacentes à Lagoa dos Patos, pois translocam milhares de indivíduos, principalmente nas localidades do entorno da Praia do Laranjal, Pelotas, Rio Grande do Sul, Brasil. Este cenário proporcionou uma oportunidade rara de amostrar um número significativo de indivíduos de maneira não seletiva, permitindo realizar uma análise bastante detalhada das populações de anfíbios e répteis ali existentes. Três capítulos formam a base dessa dissertação. O primeiro capítulo descreve e quantifica anfíbios e répteis deste evento de inundação. O segundo capítulo apresenta dados sobre estrutura populacional, proporção e dimorfismo sexual, relações peso-comprimento de machos e fêmeas, além da descrição da variação de colorido ventral de *Helicops infrataeniatus*. O terceiro capítulo descreve o primeiro caso de intersexualidade para a tribo Hydropsini, que foi descoberto após o exame minucioso da população estudada. Além destes, em anexo a esta dissertação se encontra um artigo de descrição de uma espécie de cobra-de-vidro *Ophiodes* sp. descoberta durante o mesmo evento de inundação, além de outros três artigos sobre história natural de anfíbios da região, obtidos através de resultados parciais do projeto original de mestrado.

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INTRODUÇÃO GERAL

Os Squamata são considerados a ordem mais diversa de répteis atualmente e é o segundo maior grupo de tetrápodes viventes. Esta ordem apresenta um grande polimorfismo, desde formas tetrápodes, como a maioria dos lagartos, até formas ápodas, como as serpentes e anfisbenas. Lagartos e serpentes possuem uma variada diversidade de hábitos, havendo espécies terrestres, arborícolas, aquáticas, cavadoras e fossoriais, enquanto que as anfisbenas são sempre fossoriais (Lema 2002, Quintela & Loebmann 2009). Esta grande diversidade morfológica permitiu que esse grupo ocupasse praticamente todos os ambientes e nichos aquáticos e terrestres (Quintela & Loebmann 2009, Herpetologia UFRGS 2010). Apresentam o corpo totalmente revestido por escamas, cuja forma, número e disposição são importantes caracteres taxonômicos (Lema 2002, Quintela & Loebmann 2009, Herpetologia UFRGS 2010).

Para o Rio Grande do Sul (RS), atualmente 89% da fauna reptiliana pertence à ordem Squamata, com cinco espécies de anfisbenas, 21 espécies de lagartos e 74 espécies de serpentes (Lema 1994, Quintela & Loebmann 2009, Di-Bernardo et al. 2004). Dessas, 28 espécies (uma anfisbena, oito lagartos e 19 serpentes) são registradas para a região costeira, adjacente à desembocadura da Lagoa dos Patos (municípios de Pelotas e Rio Grande) (Gomes & Krause 1982, Quintela et al. 2006, Quintela & Loebmann 2009, Entiauspe-Neto et al. 2016). Isto corresponde a 28% dos Squamata registrados para o RS e 48% dos répteis conhecidos para a planície costeira do Estado (Lema 1994, Quintela & Loebmann 2009).

Assim como ocorre em toda a região Neotropical, Diapsidae (*sensu* Grazziotin et al. 2012) é a família de serpentes mais representativa para o RS em riqueza de espécies, atualmente com 58 espécies conhecidas (Herpetologia UFRGS 2010). Os Dipsadidae do Rio Grande do Sul estão representados em 30 gêneros e 11 tribos: Dipsadini, Echinantherini, Elapomorphini, Hydrodynastini, Hydropsini, Philodryadini, Pseudoboini, Psomophini, Tachymenini, Tropidodryadini e Xenodontini (SBH 2016), além de *Uromacerina ricardinii* (Peracca 1897) atualmente sem tribo definida. Dessas, Xenodontini e Pseudoboini são as mais representativas, com 11 e 10 espécies para o Estado, respectivamente.

Estrutura populacional em Squamata

Uma população biótica é um conjunto de indivíduos de uma mesma espécie que ocupa a mesma área geográfica (Odum 1985, Ricklefs 2003). A estrutura de uma população diz respeito aos atributos desta, incluindo a densidade e a distribuição dos indivíduos na sua abrangência geográfica, e as proporções dos indivíduos em cada faixa etária e classe sexual (Ricklefs 2003). As populações bióticas frequentemente sofrem flutuações, as quais resultam principalmente de mudanças sazonais ou anuais na disponibilidade de recursos (Odum 1985).

Devido à sensibilidade dos animais ectotérmicos às flutuações ambientais, ciclos reprodutivos sazonais são comuns em répteis de ecossistemas marcados pela sazonalidade (Fitch 1980, Rocha 1998). Nos trópicos e ambientes semi-áridos a flutuação das populações é atribuída à precipitação, enquanto que em regiões de climas temperados, a temperatura tem sido apontada a como principal determinante do ciclo de vida das espécies (Licht & Gordmann 1970, Sexton et al. 1971, Fitch 1980). Isso ocorre porque o período reprodutivo da maioria das espécies coincide com as condições mais favoráveis, permitindo uma maior taxa de sobrevivência da próxima geração (Licht 1966, Licht & Gordmann 1970). A partir daí o recrutamento cai e a mortalidade e a emigração levam a um declínio populacional, até a próxima estação reprodutiva (Rocha 1998).

Na literatura, estudos referentes à estrutura populacional em Squamata ainda são limitados. Para a subordem Sauria, pesquisas relacionadas à estrutura populacional das espécies podem ser encontradas para algumas populações do Brasil (e.g. Rocha 1998, Rosumek et al. 2007). No entanto, para as anfisbenas e serpentes informações básicas sobre razão sexual, estrutura de tamanho, relação peso-comprimento, entre outras ainda são incipientes. O hábito fossorial de muitas espécies e a dificuldade de amostrar populações de serpentes em quantidade podem ser apontados como principais fatores que limitam gerar conhecimento sobre a biologia destas populações. Nesse contexto, ressalta-se a importância de coleções biológicas como uma das principais fontes de dados para esse tipo de abordagem.

Eventos de inundação: causas e consequências

Eventos de inundação são considerados fenômenos de ocorrência natural, os quais acontecem habitualmente em bacias hidrográficas, principalmente de médio e grande porte (Pompêo 2000). Esses eventos são dependentes dos processos climáticos regionais (Tucci 2003) e globais (Mechoso & Iribarren 1992, Diaz et al. 1998), ocorrendo quando a capacidade de drenagem do corpo aquático é ultrapassada.

Embora a precipitação seja um fator chave para que ocorra uma inundação, esse fenômeno é resultado de uma combinação de condicionantes naturais, incluindo características da rede de drenagem da bacia hidrográfica, formas do relevo, tipo de solo e presença ou ausência da cobertura vegetal, também condicionantes de origem antrópica como o uso e ocupação irregular nas planícies e margens dos cursos da água (Amaral & Ribeiro 2009). A intensidade das chuvas em determinada região geográfica pode também estar vinculada a fenômenos climáticos de escala global, como é o caso do *El Niño*. No sul do Brasil, Uruguai e na Argentina, a ocorrência de episódios de chuvas excessivas em determinados anos, está fortemente associado a este fenômeno, que é caracterizado pelo aquecimento anômalo das águas superficiais do Pacífico Equatorial Oriental (Ropeleswki & Halpert 1987, 1989, Grimm et al. 1998, Grimm et al. 2000).

Dentre as áreas mais susceptíveis a estes fenômenos de inundação, estão as várzeas adjacentes aos rios. Nestas áreas, devido às constantes flutuações do regime hidrológico, a biota terrestre necessita adaptar-se às pressões e às condicionantes hidrológicas. Os principais mecanismos biológicos de resposta a essa flutuação hidrológica são deixar o sistema quando as condições são adversas, produzir formas latentes que suportam o dessecamento ou serem formas resistentes a longos períodos de inundação (Rebouças et al. 2015).

O Canal São Gonçalo interliga as duas maiores lagoas do Brasil, a Lagoa dos Patos e a Lagoa Mirim, cujas bacias contribuintes recebem cerca de 70% do volume de águas fluviais do Rio Grande do Sul (Silva et al. 2007). A região apresenta topografia predominantemente plana, com altitudes de 0 à 27 m, e devido às baixas altitudes e sua localização próxima a grandes corpos da água, fica constantemente sujeito a inundações.

Desde que dados metereológicos são coletados pela Estação Agroclimatológica de Pelotas, foram detectados 13 eventos de inundações na região do Canal São Gonçalo e áreas adjacentes, que se destacam devido as suas elevadas proporções (*sensu* Hansmann 2013). O último grande evento de inundação ocorreu no ano de 2015, entre os meses de outubro e novembro, onde foi registrado, ao longo de 10 dias, uma precipitação total acumulada de 174 mm (http://agromet.cpact.embrapa.br/), resultando em um evento de inundação das regiões mais baixas do município. Foi o caso da localidade da Praia do Laranjal, localizada às margens da Lagoa dos Patos (Figura 1). Durante este evento de inundação, uma quantidade massiva de material biológico foi carreada das várzeas do canal São Gonçalo e depositada na Praia do Laranjal (Figura 2).

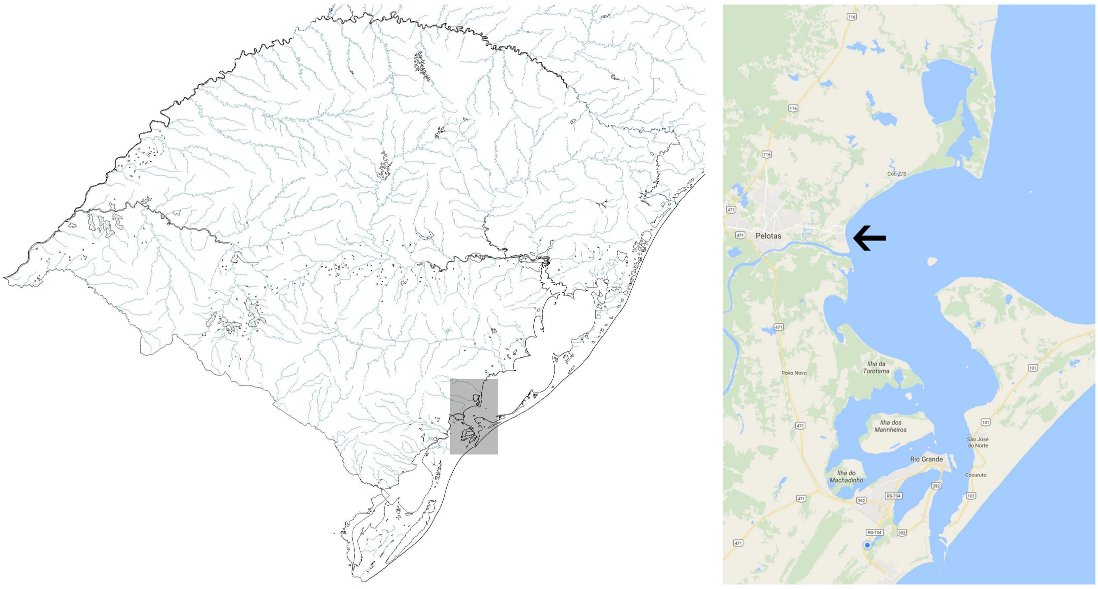

Figura 1. Localização da área de estudo. A seta indica a Praia do Laranjal, Município de Pelotas, Rio Grande do Sul, Brasil.

Figura 2. Acúmulo de material vegetal, predominantemente partes da macrófita *Typha* sp., acumulado às margens da Praia do Laranjal, Pelotas, Rio Grande do Sul, Brasil, em novembro de 2015.

Agregação em répteis

Agregação de indivíduos de uma mesma espécie é um fenômeno comumente registrado, fato este que ocorre também para grupos de répteis (Burghardt & Rand 1982; Gillingham 1987, Gregory 1984, Gregory et al. 1987). De acordo com Gregory et al. (1987), uma agregação é "qualquer concentração de indivíduos em uma área relativamente pequena, tal que a densidade de indivíduos na agregação contrasta fortemente com a de uma área circundante".

Para répteis, a maioria das agregações ocorre de forma ocasional, como por exemplo, as observadas e descritas a seguir: agregações em virtude de uma ampla oferta de alimento (presas), eventos de reprodução (Allee 1938, Arnold & Wassersug 1978, Ford & Bleness 1986, Graves & Duvall 1995), localização da água (Brown & MacLean 1983, Graves et al. 1986, Reinert & Zappalorti 1988), termorregulação (Graves & Duvall 1987, Graves et al. 1986) e defesa (Graves 1989, Graves & Duvall 1988).

Porém agregações massivas de répteis podem ainda ocorrer por circunstâncias casuais. Por exemplo, um grande número de indivíduos de um táxon pode ser carregado de forma ativa por águas de enchentes, acarretando no acúmulo destes em uma determinada região (Covacevich 1974, Lema 2002). Tanto o transporte de matéria vegetal, quanto materiais sólidos de origem humana, como plástico, podem ser um mecanismo potencial de dispersão de animas terrestres por distâncias consideráveis na água, sendo este modo de dispersão um provável fenômeno de colonização de novas áreas (Ingó1fsson 1992 e 1995, Aliani e Molcard 2003).

Reprodução em Squamata

Como todos os Ainiota, os répteis apresentam fecundação interna e, com exceção dos Tuataras, que são desprovidos de órgão copulador, os machos deste grupo apresentam um órgão copulatório denominado hemipênis (Vitt & Caldwell 2013). Enquanto a grande maioria das espécies de répteis é ovípara, algumas outras, principalmente as relacionadas aos ambientes aquáticos tendem a ser vivíparas (Lema 2002, Carreira & Maneyro 2013). Curiosamente, a espécie *Helicops angulatus* exibe ambos os modos reprodutivos (Rossman 1984, Aguiar & Di-Bernardo 2005, Braz et al. 2016). Algumas espécies de Squamata evoluíram para uma condição assexuada, sendo os únicos vertebrados que se reproduzem verdadeiramente por partenogênese (Kearney et al. 2009, Vitt & Caldwell 2013). A partenogênese predomina em lagartos (p. ex.: Darevski 1966, Hardy & Cole 1981, Schmidtler 1993, Schmidtler et al. 1994), mas também ocorre em algumas espécies de serpentes (Wynn et al. 1987, Groot et al. 2003, Booth et al. 2012).

Outra condição conhecida para répteis é a intersexualidade, definida como uma condição em que estruturas reprodutivas em um determinado sexo também são encontradas no sexo oposto (Goldschmidt 1917). Esta condição pode incluir tanto o hermafroditismo (presença de tecidos ovarianos e testiculares) como o pseudo-hemafroditismo (presença de tecido gonadal de um único sexo) (Forbes 1964). Em répteis Squamata, casos de intersexualidade estão bem relatados em lagartos (Böhme 1995, Ziegler & Böhme 1997, Ziegler et al. 1999, Telemeco 2015, Valdecantos & Lobo 2015), anfisbenas (Kasperoviczus et al. 2011) e serpentes (Hoge et al. 1954, Hoge & Penha 1959, Maclean 1968, Hardy 1970, Ziegler & Böhme 1997). A condição intersexual nestes grupos é geralmente evidenciada em fêmeas, às quais apresentam um órgão equivalente ao hemipênis masculinos, mas geralmente menores, denominados hemiclitóris (Böhme 1995).

Espécie foco do estudo

Os Hydropsini têm sido amplamente suportados na literatura como uma clado monofilético (Vidal et al. 2000, Zaher et al. 2009, Grazziotin et al. 2012, Pyron et al. 2013), atualmente representado pelos gêneros *Helicops*, *Hydrops* e *Pseudoeryx*, totalizando 22 espécies descritas (Uetz et al. 2016). O gênero Neotropical *Helicops* Wagler, 1828 é composto por 17 espécies de serpentes, com distribuição registrada da Colômbia até o sul da Argentina (Rossman 1970). Os integrantes deste táxon apresentam um conjunto de características morfológicas típicas de serpentes adaptadas ao ambiente aquático (Scartozzoni 2005), isto é, apresentam os olhos e narinas localizados na parte superior da cabeça, escamas quilhadas e viviparidade (Rabb & Marx 1973, Rossman 1974, Cei 1993).

A espécie foco neste trabalho é a cobra-d'água-pampeana, *Helicops infrataeniatus* Jan 1865 (Figura 3). Essa espécie é uma das mais abundantes e, possivelmente a que apresenta maior densidade populacional na planície costeira do Rio Grande do Sul (Quintela & Loebmann 2009). Possui distribuição conhecida do sul do Paraguai, nordeste da Argentina, sul do Brasil até o Uruguai (Deiques & Cechin 1991, Lema 1994, Rossman 2000, Giraudo 2001). Com hábitos predominantemente aquáticos esta espécie é encontrada em ambientes lóticos e lênticos, tais como arroios, canais, banhados e lagoas, com registros inclusive para valetas e canaletes em áreas urbanas (Lema 1994, Quintela & Loebmann 2009, Carreira & Maneyro 2013). Sua dieta consiste principalmente de peixes e anfíbios (Araújo & Ely 1980, Feltrim & Cechin 2000).

Indivíduos desta espécie podem chegar até um metro de comprimento (Giraudo 2001, Quintela & Loebmann 2009, Carreira & Maneyro 2013), sendo que o maior indivíduo até então registrado possui 1005 mm e o menor 170 mm (Giraudo 2001). A espécie apresenta dimorfismo sexual, sendo que as fêmeas possuem um comprimento total maior, enquanto, os machos apresentam caudas proporcionalmente maiores (Giraudo 2001, Carreira & Maneyro 2013). A reprodução é vivípara, com uma média de duas a 25 crias por ninhada e o período reprodutivo ocorre nas estações do ano da primavera e verão (Lema 2002, Quintela & Loebmann 2009, Carreira & Maneyro 2013).

Figura 3. Indivíduo de *Helicops infrataeniatus* registrado na Planície Costeira sul do Rio Grande do Sul. Foto de Daniel Loebmann.

A coloração dorsal dos indivíduos da espécie varia de castanho-escuro à verde acastanhado com faixas longitudinais negras (Giraudo 2001, Quintela & Loebmann 2009, Carreira & Maneyro 2013). Ventralmente trata-se de uma espécie polimórfica, possuindo diferentes padrões de estrias e manchas paraventrais ou transversais negras, com fundos variando entre as colorações em tons de amarelo ou vermelho (Giraudo 2001, Lema 2002, Quintela & Loebmann 2009). Estes diferentes morfotipos de coloração ventral são classificados em três padrões distintos: padrão lineado (ventre com três linhas longitudinais pretas e fundo variando entre as cores amarela, branca ou vermelha), xadrezado (ventre com escamas de coloração alternada, de preto com vermelho, branco ou amarelo) e padrão intermediário (onde a parte anterior do ventre é do tipo lineado e a parte posterior tipo xadrezado) (Boulenger 1893, 1894, Lema 2002).

Segundo Giraudo (2011), nas populações de *H. infrataeniatus* do Uruguai os diferentes morfotipos de coloração ventral (lineado, xadrezado e intermediário) apresentam distintas frequências de ocorrência nas diferentes áreas geográficas de ocorrência das populações desta espécie no país. E mesmo que ocorra sobreposição de morfotipos, há padrões de morfotipos dominantes em cada população.

Justificativas e objetivos

Obter dados referentes à estrutura populacional são ferramentas poderosas no monitoramento de populações animais (Millar & Libby 1991), pois apresentam indicadores numéricos comparáveis sobre as flutuações das espécies ao longo do tempo. A dificuldade em amostrar um grande número de indivíduos devido ao seu comportamento criptozóico e baixa densidade em estado selvagem (Fitch 1975, Sun et al. 2001) são apontados como os principais fatores que contribuem para a falta de informação sobre a biologia das espécies de répteis. De fato, estudos sobre a estrutura populacional em Squamata são raros (p. ex.: Keogh et al. 2000, Ford & Ford 2002, Ávila et al. 2006), e a maioria concentra-se em lagartos (p. ex.: Rocha 1998, Horreo & Fitze 2015). Para anfísbenas e serpentes, informações sobre estrutura populacional, relações tamanho-peso, entre outros, ainda são incipientes.

Em outubro de 2015, após 10 dias consecutivos de chuva, a Praia do Laranjal e áreas adjacentes foram inundadas. Esse evento relacionado ao fenômeno climático do El Niño, resultou no carreamento de milhares de indivíduos de anfíbios e répteis, que se concentraram ao longo de um trecho de aproximadamente 1000 m de linha de praia. Este cenário proporcionou uma oportunidade rara de amostrar um número grande de indivíduos e de maneira não seletiva, proporcionando uma "fotografia" fideldigna da estrutura das populações de anfíbios e répteis ali existentes. Essa oportunidade permitiu três frentes importantes, a saber: ter uma amostra representativa da cobra-d'água *Helicops infrataeniatus* para analisar dados populacionais, amostrar uma quantidade significativa da cecília *Chthonerpeton indistinctum* e descobrir uma espécie não descrita de cobra-de-vidro *Ophiodes* sp.

Assim, o foco desta dissertação é apresentar dados da biologia de *H. infrataeniatus*. O primeiro capítulo descrever e quantificar anfíbios e répteis do evento de inundação. No segundo capítulo são apresentados dados inéditos sobre estrutura populacional, proporção e dimorfismo sexual, relações peso-comprimento de machos e fêmeas, além da descrição da variação de colorido ventral. No terceiro capítulo é descrito o primeiro caso de intersexualidade para a tribo Hydropsini, que foi descoberto após o exame minucioso da população estudada.

Bibliografia

Aguiar, L.F.S. & Di-Bernardo, M. (2005). Reproduction of the water snake *Helicops infrataeniatus* (Colubridae) in southern Brazil. *Amphibia-Reptilia* **26**: 527-533.

Aliani, S. & Molcard, A. (2003). Hitch-hiking on loating marine debris: macrobenthic species in the Western Mediterranean sea. *Hydrobiologia* **503**: 59-67.

Allee, W.C. (1938). *The Social Life of Animals*. 1° Ed. W. W. Norton & Company Inc., New York, 293p.

Amaral, R. & Ribeiro, R.R. (2009). Enchentes e inundações. pp. 40-53. In: Tominaga, L.K., Santoro, J., Amaral, R. (Eds.). *Desastres naturais, conhecer para prevenir*. Instituto Geológico, São Paulo.

Araújo, M.L. & Ely, L. (1980). Incidência sazonal de *Helicops carinicaudus infrataeniatus* (Jan, 1865) e *Philodryas patagoniensis patagoniensis* (Girard, 1857) no estado do Rio Grande do Sul, Brasil (Ophidia-Colubridae). *Iheringia Série Zoologica* **55**: 87-106.

Arnold, S.J. & Wassersug, R.J. (1978). Differential predation on metamorphic anurans by garter snakes (*Thamnophis*): social behavior as a possible defense. *Ecology* **59**: 1014-1022.

Böhme, W. (1995). Hemiclitoris discovered: a fully differentiated erectile structure in female monitor lizards (*Varanus* spp.) (Reptilia: Varanidae). *Journal of Zoological Systematics and Evolutionary Research* **33**: 129-132.

Booth, W., Smith, C.F., Eskridge, P.H., Hoss, S.K., Mendelson, J.R. & Schuett, G.W. (2012). Facultative parthenogenesis discovered in wild vertebrates. *Biology Letters* **8**: 983-985.

Boulenger, A. (1893). *Catalogue of the Snakes in the British Museum (Natural History).* Vol. I. London, 448 pp.

Boulenger, A. (1894). *Catalogue of the Snakes in the British Museum (Natural History).* Vol. II. London, 382 pp.

Braz, H.B., Scartozzoni, R.R. & Almeida-Santos, S.M. (2016). Reproductive modes of the South American water snakes: A study system for the evolution of viviparity in squamate reptiles. *Zoologischer Anzeiger* **263**: 33-44.

Brown, W.S. & MacLean, F.M. (1983). Conspecific scent-trailing by newborn timber rettlesnakes, *Crotalus horridus*. *Herpetologica* **39**: 430-436.

Burghardt, G.M. & Rand, A.S. (1982). *Iguanas of the World: Their Behavior, Ecology, and Conservation.* Noyes Publications, Park Ridge, New Jersey.

Carreira, S. & Maneyro, R. (2013). *Guía de Reptiles del Uruguai*. Ediciones de la Fuga. 285p.

Cei, J.M. (1993). *Reptiles del noroeste y este de la Argentina. Herpetofauna de las Selvas subtropicales, Puna y Pampas*. Torino - Museo Regionale di Scienze Naturali, 949 pp.

Covacevich, J. (1974). An unusual aggregation of snakes following major flooding in the Ipswich - Brisbane area, south-eastern Queensland. *Herpetofauna* **7**: 21-24.

Darevski, I.S. (1966). Natural Parthenogenesis in a Polymorphic Group of Caucasian Rock Lizards Related to *Lacerta saxicola* Eversmann. *Journal of the Herpetological Society* **5**: 115-152.

Deiques, C.H. & Cechin, S. (1991). O status de *Helicops carinicaudus* (Wied, 1825) (Serpentes - Colubridae). *Acta Biologica Leopoldensia* **12**: 313–326.

Diaz, A.F, Studzinski, C.D. & Mechoso, C.R. (1998). Relationships between precipitation anomalies in Uruguay and southern Brazil and sea surface temperature in the Pacific and Atlantic Oceans. *Journal of Climate* **11**: 251-271.

Di-Bernardo, M., Borges-Martins, M. & Oliveira, R.B. (2004). Proposed deletion of eight species of snakes from the Brazilian State of Rio Grande do Sul herpetofauna. *Comunicações do Museu de Ciências e Tecnologia da PUCRS, Série Zoologia* **17**: 45-50.

Entiauspe-Neto, O.M., Perleberg, T.D. & Freitas, M.A. (2016). Herpetofauna from an urban Pampa fragment in southern Brazil: Composition, Structure and Conservation. *Check List* **12**: 1-15.

Feltrim, A. & Cechin, S. (2000). Natural history notes: *Helicops infrataeniatus* (NCN). *Herpetological Review* **31**: 1-46.

Ficth, H.S. (1970). Reproductive strategies of reptiles. pp. 25-31. In: Murphy, J.B. & Collins, J.J. (Eds.). *Reproductive biology and diseases of captive reptiles*. 1° Ed. New York, SSAR, Contribuitions in Herpetology.

Forbes, T. R. (1964). Intersexuality in reptiles. In *Intersexuality in vertebrates including man*, 273-284. Armstrong, C. N. & Marshall, A. J. (Eds.). Academic Press, New York.

Ford, N.B. & O'Bleness, M.L. (1986). Species and Sexual Specificity of Pheromone Trails of the Garter Snake, *Thamnophis marcianus*. *Journal of Herpetology* **20**: 259-262.

Gillingham, J.C. (1987). Social behavior. pp. 184-209. In: Seigel, R.A., Collins, J.T. & Novak, S.S. (Eds.). *Snakes - Ecology and Evolutionary Biology*. McGraw-Hill, New York.

Giraudo, A.R. (2001). *Serpientes de la Selva Paranaense y del Chaco Húmedo*. Literature of Latin America, Buenos Aires, Argentina, 328p.

Goldschmidt, R. (1917). Intersexuality and the endocrine aspect of sex. *From the Osborn Zoological Laboratory, Yale University* **1**: 433-456.

Gomes, N. & Krause, L. (1982). Lista preliminar de répteis da estação ecológica do Taim, Rio Grande do Sul. São Paulo. *Revista Brasileira de Zoologia* **1**: 71-77.

Gaves, B.M. (1989). Defensive behavior of female prairie rattlesnakes (*Crotalus viridis*): Chamges after parturition. *Copeia* **1989**: 791-794.

Graves, B.M. & Duval, D. (1987). An experimental study of aggregation and thermoregulation in prairie rattlesnakes (*Crotalus vividis vividis*). *Herpetologica* **43**: 253-258.

Gaves, B.M. & Duvall, D. (1988). Evidence of an alarm pheromone from the cloacal sacs of prairie rattlesnakes. South west. *Nature* **33**: 339-345.

Graves, B.M., Duvall, D., King, M.B., Lindstedt, S.L. & Gern, W.A. (1986). Initial den location by neonatal prairie rattlesnakes: Functions, causes and natural history in chemical ecology. pp. 285-304. In: Duvall, D., Muller-Schwarze, D. & Silverstein, R.M. (Eds.). *Chemical Signals in Vertebrates* 4. Plenunm, New York.

Graves, B.M. & Duvall, D. (1995). Aggregation of squamate reptiles associated with gestation, oviposition, and parturition. *Herpetological Monographs* **9**: 102-119.

Grazziotin, F.G., Zaher, H., Murphyc, R.W., Scrocchi, G., Benavides, M.A., Zhangd Y.P. & Bonatto S.L. (2012). Molecular phylogeny of the New World Dipsadidae (Serpentes: Colubroidea): a reappraisal. *Cladistics* **1**: 1-23.

Gregory, P.T. (1984). Communal denning in snakes. pp. 57-75. In: Seigel, R.A., Hunt, L.E., Knight, J.L., Malaret, L. & Zuschlag, N.L. (Eds.). *Vertebrate Ecology and Systematics - A tribute to Henry S. Fitch*. Museum of Natural History of the University of Kansas, Lawrence.

Gregory, P.T., Macartney, J.M. & Lar-Sem, K.W. (1987). Spatial patterns and movements. pp. 366-395. In: Seigel, R.A., Collins, J.T. & Novak, S.S. (Eds.). *Snakes: ecology and evolutionary biology*. McGraw-Hill, New York.

Grimm, A.M., Ferraz, S.E.T. & Gomes, J. (1998). Precipitation anomalies in southern Brazil associated with *El Niño* and *La Niña* events. *Journal of Climate* **11**: 2863-2880.

Grimm, A.M., Barros, V.R., & Doyle, M,E. (2000). Climate variability in southern South America associated with *El Niño* and *La Niña* events. *Journal of Climate* **13**: 35-58.

Groot, T.V.M., Bruins, E. & Breeuwer, J.A.J. (2003). Molecular genetic evidence for parthenogenesis in the *Burmese python*, *Python molurus bivittatus*. *Heredity* **90**: 130-135.

Hansmann, H.Z. (2013). Descrição e Caracterização das Principais Enchentes e Alagamentos de Pelotas, RS. Trabalho de Conclusão de Curso, Pelotas, 62p. Disponível em: <ttp://wp.ufpel.edu.br/esa/files/2013/10/TCC-HENRIQUE-HANSMANN1.pdf.>. Acesso em 01 de novembro de 2016.

Hardy, L.M. (1970). Intersexuality in a Mexican Colubrid Snake (*Pseudoficimia*). *Herpetologica* **26**: 336-343.

Hardy, L.M. & Cole, C.J. (1981). Parthenogenetic reproduction in lizards: histological evidence. *Journal of Morphology* **170**: 215–237.

Herpetologia UFRGS. (2010). Laboratório de Herpetologia da Universidade Federal do Rio Grande do Sul. Versão 1.0, online, Novembro 2010. Disponível em: <http://www.ufrgs.br/herpetologia>. Acesso em 30 de novembro de 2016.

Hoge, A., Belluomini, H. & Schreiber, G. (1954). Intersexuality in a highly isolated population of snakes. Atti *del IX Congresso Internazionale di Genetica, Caryologica, Vol. suppl. p.* 964-965.

Hoge, A. & Penha, A.M. (1959). Sexual abnormalities in *Bothrops insularis* (Amaral) 1921. *Memórias do Instituto Butantan* **29**: 17-88.

Ingólfsson, A. (1992). The origin of the rocky shore fauna of Iceland and the Canadian Maritimes. *Journal of Biogeography* **19**: 705-712.

Ingólfsson, A. (1995). Floating clumps of seaweed around Iceland: natural microcosms and means of dispersal for fauna. *Marine Biology* **122**: 13-21.

Kaspervoviczus, K.N., Santos, L.C. & Almeida-Santos, S. (2011). First report of hemiclitores in a female of the amphisbaenian *Amphisbaena microcephala* (Wagler, 1824). *Herpetology notes* **4**: 41-43.

Kearney, M., Fujita, M.K., & Ridenour, J. (2009). Lost Sex in the Reptiles: Constraints and correlations. In *Lost Sex - The Evolutionary Biology of Parthenogenesis,* 447-474. Schön, I., Martens, K. & van Dijk, P. (Eds.). Springer, Dordrecht, Heidelberg, London, New York.

Lema, T. (1994). Lista comentada dos répteis do Rio Grande do Sul. *Comunicações do Museu de Ciências Tecnológicas PUCRS, Série Zoologia* **7**: 41-150.

Lema, T. (2002). *Os Répteis do Rio Grande do Sul. Atuais e Fósseis- Biogeografia - Ofidismo*. Porto Alegre, Edipucrs, 264p.

Licht, P. (1966). Reproduction in lizards: influence of temperature on photoperiodism in testicular recrudescense. *Science* **154**: 1668-1670.

Licht, P. & Gordmann, G.C. (1970). Reproductive and fat cycles in Caribbean *Anolis* lizards. *University of California Publications in Zoology* **95**: 1-52.

Maclean, W.P. (1968). A case of intersexuality in *Bothrops moojeni* Hoge. *Copeia* **1968**: 170.

Mechoso, C.R., & Iribarren, G.P. (1992). Streamflow in southeastern South America and the Southern Oscillation. *Journal of Climate* **5**: 1535-1540.

Odum, E.P. (1985). *Ecologia.* Tradução por Tribe, C.J., Rio de Janeiro. Editora Guanabara Koogan, 434p.

Pompeo, A.C. (2000). Drenagem Urbana Sustentável. *Revista Brasileira de Recursos Hídricos* **5**: 15-23.

Pyron, R.A., Burbrink, F.T. & Wiens, J. (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology* **19**: 1-93.

Quintela, F.M., Loebmann, D. & Gianuca, N.M. 2006. Répteis continentais do município de Rio Grande, Rio Grande do Sul, Brasil. *Biociências* **14**: 180-188.

Quintela, F.M. & Loebmann, D. 2009. *Guia ilustrado - Os répteis da região costeira do extremo sul do Brasil.* Useb, Pelotas, RS.

Rabb, G.B. & Marx, H. (1973). Major ecological and geographic pattern in the evolution colubroid snakes. *Evolution* **27**: 69-83.

Rebouças, A.C., Braga, B. & Tundisi, J.G. (2015). *Águas doces no Brasil: capital ecológico, uso e conservação*. 4° Ed. Editora Escrituras 732p.

Reinert, H.K. & Zappalorti, R.T. (1988). Timber rattlesnakes (*Crotalus horridus*) of the Pine Barrens: Their movement patterns and habitat preference. *Copeia* **1988**: 964-978.

Ricklefs, R.E. (2003). *A economia da natureza.* 5° Ed. Editora Guanabara Koogan, Rio de Janeiro.

Rocha, C.F.D. (1998). Population dynamics of the endemic tropidurid lizars *Liolaemus lutzae* e seaseazonal restinga habitat. *Ciência e Cultura* **50**: 446-451.

Rosumek, F.B., Faria, P.E.P., Mozele, H.B., Matos, J.J. & Graipel, M.E. (2007). Dinâmica populacional de *Liolaemus occipitalis* (Squamata: Tropiduridae) nas dunas das praias da Joaquina, Ilha de Santa Catarina, Sul do Brasil. Anais do VIII Congresso de Ecologia do Brasil, Caxambu, MG. Disponível em: <http://www.seb-ecologia.org.br/viiiceb/pdf/961.pdf.> Acesso em 22 de novembro de 2016.

Ropelewski, C.F., & Halpert, M.S. (1987). Global and regional scale precipitation patterns associated with the *El Niño*/Southern Oscillation. *Monthly Weather Review* **115**: 1606-1626.

Ropelewski, C.F., & Halpert, M.S. (1989). Precipitation patterns associated with the high index phase of the southern oscillation. *Journal of Climate* **2**: 268-284.

Rossman, D.A. (2000). Identity of the snake *Calopisma septemvittatum* Fischer, 1879, and redescription of the lectotype of *Helicops infrataeniatus* Jan, 1865. *Mitteilungen aus dem Hamburgischen Zoologychen Museum und Institut* **97**: 123-129.

Rossman, D.A. (1970). *Helicops* Wagler. pp. 122-125. In: Peters, J.A., & Miranda, O. (Eds.). *Catalogue of the Neotropical Squamata*. Part. I. Snakes. United States National Museum Bulletin.

Rossman, D.A. (1974). Miscellaneous notes on the South American water snake genus *Helicops*. *Herpetological Information Search Systems News-Journal* **1**: 189-191.

Rossman, D.A. (1984). *Helicops angulatus* (South American Water Snake). Reproduction. *Herpetological Review* **15**: 50.

SBH (2016). Brazilian reptiles - List of species. Disponível em: http://www.sbherpetologia.org.br. Sociedade Brasileira de Herpetologia. Acesso em 24 de novembro de 2016.

Scartozzoni, R.R. (2005). Morfologia de serpentes aquáticas neotropicais: um estudo comparativo. Dissertação de Mestrado. Universidade de São Paulo, SP. Disponível em:<http://www.ecoevo.com.br/publicacoes/alunos/rodrigo_scartozzoni/Rodrigodissertacao_2005.pdf.>. Acesso em 15 de dezembro de 2016.

Sexton, O.J., Ortleb, E.P., Hathaway, L.M. & Ballinger, R.E. (1971). Reproductive eyeles of tree species of anoline lizards from the Isthmus of Panamá. Wasshington. *Ecology* **52**: 201-212.

Silva, C.S. (2007). Inundações em Pelotas/RS: O uso de geoprocessamento no planejamento paisagístico ambiental. Dissertação de Mestrado, Santa Catarina 126p. Disponível em: <file:///C:/Users/Ruth%20Regnet/Downloads/256550.pdf.>. Acesso em 26 de dezembro de 2016.

Schmidtler, J. F. (1993). Parthenogenetische Felseideshsen aus de Ost-Türkei. *Die Eidechse*. **8**: 3-5.

Schmidtler, J. F., Eiselt, J. & Darevsky, I. S. (1994). Untersuchungen an Felseidechsen (Lacerta saxkola-Gruppe) in der östlichen Türkei: 3. Zwei neue parthenogenetische Arten. *Salamandra* **30**: 55-70.

Telemeco, R. S. (2015). Sex Determination in Southern Alligator Lizards (*Elgaria multicarinata*; Anguidae). *Herpetologica* **71**: 8-11.

Tussi, C.E.M. (2003). Inundações e Drenagem Urbana. pp. 45-129. In: Tucci, C.E.M. & Bertoni, J.C. (Eds.). *Inundações Urbanas na América do Sul.* Porto Alegre: Associação Brasileira de Recursos Hídricos.

Uetz, P., Freed, P. & Hosek, J. (Eds.). The Reptile Database. Disponível em: <http://www.reptiledatabase.org/>. Acesso em 17 de novembro de 2016.

Valdecantos, S.; Lobo, F. (2015). First Report of Hemiclitores in Females of South American Liolaemid Lizards. *Journal of Herpetology* **49**: 291-294.

Vidal, N., Kindl, S.G., Wong, A. & Hedges, S.B. (2000). Phylogenetic relationships of xenodontine snakes inferred from 12S and 16S ribosomal RNA sequences. *Molecular Phylogenetics and Evolution* **14**: 389-402.

Villwock, J.A., Tommazelli, L.J., Loss, E.L., Dehnhard, E.A., Horn-Filho N.O., Bachi, F.A. & Dehnhardt, B.A. (1986). Geology of the Rio Grande do Sul coastal province. pp. 79-97. In: Rabassa, J. (Ed.). *Quaternary of South America and Antartic Peninsula*. Vol. 4. Balkema, Rotterdam.

Vitt, L.J. & Caldwell, J.P. (2013). *Herpetology: an introductory biology of amphibians and reptiles*. Academic Press.

Wynn, A., Charles, H., Cole, J. & Gardner. A.L. (1987). Apparent triploidy in the unisexual brahminy blind snake, *Ramphotyphlops braminus*. *American Museum Novitates* **2868**: 1-7.

Zaher, H., Grazziotin, F.G., Cadle, J.E., Murphy, R.W., Moura-Leite, J.C. & Bonatto, S.L. (2009). Molecular phylogeny of advanced snakes (Serpentes: Caenophidia) with an emphasis on South America xenodontinaes: a revised classification and descriptions of new taxa. *Papeis Avulsos de Zoologia* **49**: 115-153.

Ziegler, T., Böhme, W. (1997). Genitalstrukturen und Paarungsbiologie bei squamaten Reptilien, speziell den Platynota, mit Bemerkungen zur Systematik. *Mertensiella, Supplement zu Salamandra* **8**: 1-207.

Ziegler, T., Böhme, W. & Philipp, K.M. (1999). *Varanus caerulivirens* sp. n., a new monitor lizard of the *V. indicus* group from Halmahera, Moluccas, Indonesia (Squamata: Sauria: Varanidae. *Herpetozoa* **12**: 45-56.

CAPÍTULO 1

Manuscrito aceito para publicação no periódico Salamandra - German Journal of Herpetology. Normas do periódico encontram-se no anexo dois desta dissertação.

Título: Remarkable aggregations of squamates and caecilians associated with flood events during El Niño in southern Brazil

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Número de palavras: 1.819

Figuras: 1

Qualis Capes 2017: B1

Fator de impacto: 1,25

Remarkable aggregations of squamates and caecilians associated with flood events during El Niño in southern Brazil

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Abstract. The effects of the El Niño Southern Oscillation (ENSO) on the biota still remains poorly understood, although it is known that this atmospheric-oceanic phenomenon can influence both aquatic and terrestrial lifeforms worldwide. Herein, we report on a massive concentration of thousands of Squamata reptiles and caecilians which were transported after a period of heavy rainfall. We performed a linear transect of 1,000 m in length and 25 m in width on a tourist beach of the Lagoa dos Patos, southern Brazil during five days consecutively. A total of 2152 amphibians and reptiles were registered, 2002 of them belonging to the Pampean Water Snake *Helicops infrataeniatus.* Similar events of inundation with massive transportation of herpetofauna elements in this region occurred at least four times in the past decades, all of them associated with El Niño years.

Animal aggregation can be defined as "any concentration of individuals in a relatively small area such that density of individuals in the aggregation contrasts sharply with that in the surrounding area" (Gregory et al. 1987). For amphibians and reptiles, most aggregations occur occasionally and have been associated with high food availability (e.g. Arnold & Wassersug 1978), reproduction events (Arnold & Wassersug 1978, Ford & Bleness 1986, Graves & Duvall 1995), water availability (Graves et al. 1986, Reinert & Zappalorti 1988), thermoregulation (Graves et al. 1986) and defense (Graves 1989, Graves & Duvall 1988). However, massive reptile aggregations may also occur by incidental circumstances. For example, a large number of individuals of snakes can be actively transported by flood waters, resulting in the accumulation of these in a certain region (Lema 2002).

The effects of El Niño Southern Oscillation (ENSO) on biota are poorly known, but have shown to negatively influence both aquatic and terrestrial lifeforms (Glantz 2001). This has received special attention since the last ENSO event has been classified as the strongest ever recorded (Schiermeier 2015). In southernmost Brazil, El Niño events are associated to increased rainfall periods (Grimm et al. 2000). Recently, the lowlands surrounding the Lagoa dos Patos, the world's largest choked lagoon, located along of the middle coast of state of Rio Grande do Sul, were severely flooded, prompting a massive movement and possibly translocation of snakes, which is herein described.

In October 2015, after 10 days of consecutive rainfall (Accumulated precipitation = 174 mm, according to the Weather Station of Pelotas), an unusually large amount of amphibians, lizards and snakes was reported at the margins of the Patos Lagoon (Figure 1), causing concern to most residents of these areas and alerting official authorities (Brazilian Law Enforcement and Wildlife Services). The amphibians and reptiles arrived at the beach once that rainfall elevated the level of the Canal São Gonçalo, a waterway that connecting the Patos and Mirim lagoons, transporting a great volume of grass (*Typha* sp., predominantly), which was accumulated as patches of vegetation along the shoreline (Figure 1). In order to quantify this invasion, we conducted a transect of 1,000 m of linear length and 25 m of width was established along the coast in Praia de Laranjal (31°46'S, 52°13'W, ca. 2 m of altitude), a tourist beach in the Patos Lagoon estuary, municipality of Pelotas, state of Rio Grande do Sul, Brazil, totalizing five days of field sampling. Voucher specimens were deposited at the Herpetological Collection of Rio Grande Federal University. Collection permits was authorized by ICMBio (proc. 43658). Specimens were euthanized with an intracelomatic injection of barbituric at lethal dose (>50 mg/kg).

During the five days of field collections a total of 2014 snakes were captured, distributed into five species *Erythrolamprus jaegeri* (*n*=2), *Erythrolamprus semiaureus* (*n*=2), *Helicops infrataeniatus* (*n*=2002), *Philodryas patagoniensis* (*n*=6), and *Thamnodynastes hypoconia* (*n*=2). Surprisingly, most specimens (99.5%) were the Pampean water snake, *Helicops infrataeniatus* Jan, 1865. Individuals of *Chtonerpeton indistinctum* (Amphibia, Gymnophiona) (*n*=99) and an undescribed species *Ophiodes* (Squamata: Diploglossinae) (*n*=40) were also encountered during the event. While the snakes species recorded are common in the study area, the cryptic habits of *C. indistinctum* and *Ophiodes* sp. do not allow to infer if these species are rare in the nature or hard to be sampled instead.

Aggregations of snakes associated to flood events are poorly reported in the literature (e.g. Covacevich 1974, Lema 2002). For southern Brazil, those events seem to be associated to ENSO events. In the Lagoa dos Patos, snake aggregations were reported in 1941 (Lema 2002). Besides current data, similar events were observed for the years of 1997/98 and 2004 (F. M. Quintela & D. Loebmann unpubl. data). All these aggregation events in southern Brazil occurred in ENSO years. The observed low species diversity within aggregations could be associated to certain habitat preferences, since these species occur in estuarine lowlands and therefore might be more susceptible to floodings caused by ENSO. We assume that these aggregation events may occur recurrently and be strongly correlated with the ENSO phenomenon.

The occurrence of island species closely related to continental ones or even the presence of the same species in both environments is undoubt one of most exciting fields of research for biogeographers worldwide. The dispersion by rafts of vegetation has been pointed out as an important way to promotes entrance pulses of continental fauna on the islands. However, there are few concrete evidence to infer that viable populations of terrestrial vertebrates can be transported by water and settle in new environments. One of the most remarkable examples is reported by Censky et al (1998), which describes the simultaneous arrival of several individuals of *Iguana iguana* (Linnaeus, 1758) after a hurricane on the beaches of the Caribbean island Anguilla. In the past years, the use of molecular tools have providing insights on the mechanisms of island colonization as well as their radiations, including examples of amphibians (Vences et al. 2003; Measey et al. 2007, Stoelting et al. 2014), lizards (Mausfeld et al. 2002; Jesus et al. 2005a, b; Townsend et al. 2010) and snakes (Jesus et al. 2009). Therefore, our findings of massive translocation of herpetofauna's brings another new evidence that amphibian and reptile populations, including fossorial forms, may colonize new areas with viable populations, by dispersion through the water.

Acknowledgments

We thanks I.S. Vaz for the assistance in the field and the transport of specimens.

References

Arnold, S. J. & R. J. Wassersug (1978): Differential predation on metamorphic anurans by garter snakes (*Thamnophis*): Social behavior as a possible defense. - Ecology, **59**: 1014-1022.

Censky, E.J., K. Hoedge & J. Dudley (1998): Over-water dispersal of lizards due to hurricanes. – Nature, **395**: 556.

Covacevich, J. (1974): An unusual aggregation of snakes following major flooding in the Ipswich - Brisbane area, south-eastern Queensland. - Herpetofauna, **7**: 21-24.

Ford, N. B. & M. L. O'Bleness (1986): Species and sexual specificity of pheromone trails of the garter snake, *Thamnophis marcianus*. - Journal of Herpetology, **20**: 259-262.

Glantz, M. H. (2001): Currents of Change: Impacts of *El Niño* and *La Niña* on Climate and Society. - First edition, Cambridge, Cambridge University Press, 269 pp.

Graves, B. M., D. Duvall, M. B. King, S. L. Lindsted & W. A. Gern (1986): Initial den location by neonatal prairie rattlesnakes: Functions, causes and natural history in chemical ecology. - pp. 285-304 in: Duvall, D., D. Muller-Schwarze & R. M. Silverstein (eds): Chemical Signals in Vertebrates 4, - Plenunm press, New York.

Graves, B. M. & D. Duvall (1988): Evidence of an alarm pheromone from the cloacal sacs of prairie rattlesnakes. South west. - Nature, **33**: 339-345.

Graves, B. M. (1989): Defensive behavior of female prairie rattlesnakes (*Crotalus viridis*): Changes after parturition. - Copeia, **1989**: 791-794.

Graves, B. M. & D. Duvall (1995): Aggregation of squamate reptiles associated with gestation, oviposition, and parturition. - Herpetological Monographs, **9**: 102-119.

Gregory, P., J. Macartney & K. Larsen (1987): Spatial patterns and movements. - pp. 184-209 in: Seigel, R.A., J. T. Collins & S. S. Novak (eds): Snakes: ecology and evolutionary biology. - MacMillan press, New York.

Grimm, A. M., V. R. Barros & M. E. Doyle (2000): Climate variability in Southern America associated with *El Niño* and *La Niña* events. - Journal of Climate, **13**: 35-58.

Jesus, J., A. Brehm, D. Harris (2005a): Phylogenetic relationships of *Hemidactylus* geckos from the Gulf of Guinea islands: patterns of natural colonizations and anthropogenic introductions estimated from mitochondrial and nuclear DNA sequences. - Molecular Phylogenetics and Evolution, **34**: 480-485.

Jesus, J., A. Brehm, D. Harris (2005b): Phylogeography of *Mabuya maculilabris* (Reptilia) from São Tomé Island (Gulf of Guinea) inferred from mtDNA sequences. - Molecular Phylogenetics and Evolution, **37**: 503-510.

Jesus, J., Z. T. Nagy, W. R. Branch, M. Wink, A. Brehm & D. J. Harris (2009): Phylogenetic relationships of African green snakes (genera *Philothamnus* and *Hapsidophrys*) from São Tomé, Príncipe and Annobon islands based on mtDNA sequences, and comments on their colonization and taxonomy. - Herpetological Journal, **19**: 41-48.

Lema, T. (2002): Os Répteis do Rio Grande do Sul. Atuais e Fósseis-Biogeografia-Ofidismo. - First Edition, Porto Alegre, Edipucrs press, 264 pp.

Mausfeld, P., A. Schmitz, W. Böhme, B. Misof, D. Vrcibradic & C.F.D. Rocha (2002): Phylogenetic affinities of *Mabuya atlantica* Schmidt, 1945, endemic to the Atlantic Ocean Archipelago of Fernando de Noronha (Brazil): Necessity of partitioning the genus *Mabuya* Fitzinger, 1826 (Scincidae: Lygosominae). - Zoologischer Anzeiger - A Journal of Comparative Zoology, **241**: 281-293.

Measey, G. J., M. Vences, R. C. Drewes, Y. Chiari, M. Melo & B. Bernard (2007): Freshwater paths across the ocean: molecular phylogeny of the frog *Ptychadena newtoni* gives insights into amphibian colonization of oceanic islands. - Journal of Biogeography, **34**: 7-20.

Reinert, H. K. & R. T. Zappalorti (1988): Timber rattlesnake (*Crotalus horridus*) of pine barrens: their movement patterns and habitat preference. - Copeia, **1988**: 964-978.

Schiermeier, Q. (2015): Hunting the Godzilla *El Niño*. - Nature, **526**: 490-491.

Stoelting, R. E., G.J. Measey & R.C. Drewes (2014): Population genetics of the São Tomé Caecilian (Gymnophiona: Dermophiidae: *Schistometopum thomense*) reveals strong geographic structuring. **-** Plos One, 9:e104628.

Townsend, T.M., K.A. Tolley, F. Glaw, W. Böhme & M. Vences (2010): Eastward from Africa: palaeocurrent-mediated chameleon dispersal to the Seychelles Islands. **-** Biology Letters, **7**: 225-228.

Vences, M., D. R. Vieites, F. Glaw, H. Brinkmann, J. Kosuch, M.Veith & A. Meyer (2003): Multiple overseas dispersal in amphibians. **-** Proceedings of the Royal Society of London Biological Sciences. **270**: 2435-2442.

Figure 1. Ventral view of individuals of *Helicops infrataeniatus* captured at Laranjal Beach, municipality of Pelotas, Rio Grande do Sul, Brazil.

CAPÍTULO 2

Manuscrito submetido para o periódico Amphibia-Reptilia. Normas de formatação do periódico encontram-se no anexo três desta dissertação.

Título: Population structure and ventral polychromatism of *Helicops infrataeniatus* Jan, 1883 (Serpentes, Dipsadidae) in subtropical Brazil

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Número de palavras: 4286

Figuras: 6

Tabela: 1

Qualis Capes 2017: A2

Fator de impacto: 1,396

Population structure and ventral polychromatism of *Helicops infrataeniatus* Jan, 1883 (Serpentes, Dipsadidae) in subtropical Brazil

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Type of manuscript: Article

Total number of words: 4286

Abstract: We determine the demographic structure, sexual proportion, sexual dimorphism, and ventral polymorphism in a population of *Helicops infrataeniatus*. Specimens were collected in the municipality of Pelotas, Brazil (31°46'S, 52°13'W). From the 720 collected individuals 45.4% were females, 52.2% were males, and 2.4% were of unidentifiable sex. Population structure was composed by 51.09% adult individuals, 47.59% juveniles and 1.31% newborns. The total length (ToL) of females ranged from 18.40 to 78.20 cm; Snout-vent length (SVL) ranged from 13.30 to 62.50 cm; Tail length (TaL) ranged from 4 to 18.5 cm; Sub-caudal scales ranged from 55 to 73; and weight ranged from 2.18 to 191.02 g. ToL of males ranged from 19.70 to 67 cm; SVL ranged from 13.90 to 46.70 cm; TaL ranged from 4.90 to 21 cm; sub-caudal scales ranged from 74 to 92; and weight ranged from 2.30 to 61.27 g. Non-parametric t-tests detected highly significant differences between treatments tested (females vs males), suggesting sexual dimorphism both in the number of sub-caudal scales and in the TaL:ToL ratios. Length-weight relationships analysis detected high determination coefficients for females ($r^2 = 0.98$) and males ($r^2 = 0.97$). The intercept a was -3.22 for females and -2.93 for males, while estimates of parameter b was 2.85 and 2.60 for females and males, respectively. The frequency of ventral staining patterns was 38.39% for checkered, 35.83% for trilinear, and 25.64% for intermediate patterns. Approximately 62% of the analyzed snakes had reddish ventral coloration, 26% yellowish and 12% with both tones.

Key words: Hydropsini; length-weight relationships; sexual dimorphism; Squamata.

Introduction

Data regarding population structure have been pointed as powerful tools in monitoring animal populations (Millar and Libby, 1991), since it presents comparable numerical indicators on species fluctuations through time. Even so, studies on population structure of

Squamata are rare (e.g. Keogh et al. 2000; Ford and Ford, 2002; Ávila, Ferreira and Arruda, 2006), and most focus on lizards (e.g. Rocha, 1998; Horreo and Fitze, 2015). For amphisbeans and snakes, there are lack of information on sex ratio, size structure, size-weight relationships, among others. Difficulty to sample high numbers of individuals due to their cryptozoic behavior and low density in the wild (Fitch, 1975; Sun et al. 2001) have been pointed as the main factors that contribute to the lack of information on species biology.

Helicops currently comprises a genus of 17 recognized species with the group of water snakes of widest distribution in Neotropics (Costa et al., 2016). The Pampean water snake *Helicops infrataeniatus* occurs in southern Paraguay, northeastern Argentina, Uruguay, and southern and southeastern Brazil (Giraudo, 2001). In the coastal plain of Rio Grande do Sul, southernmost Brazil, *H. infrataeniatus* is considered one of the most abundant species (Quintela and Loebmann, 2009; Regnet et al. 2017). *Helicops infrataeniatus* is also known for its ventral polychromatism with different ventral coloration and staining patterns in the same population (see Deiques and Cechin 1991; Lema, 2002; Quintela and Loebmann, 2009).

No ecological data regarding population structure is available for the Pampean water snake *H. infrataeniatus*. Therefore, we present data on population parameters of the species in Subtropical Brazil. This represents the first large database for the species and a useful tool for monitoring the fluctuations of population attributes across time.

Materials and Methods

To conduct this study we used specimens available at the Herpetological Collection of Rio Grande Federal University (CHFURG). We selected individuals which were collected in a flood event in October 2015 at Laranjal beach, a municipality of Pelotas in the state of Rio Grande do Sul, Brazil (31°46'S, 52°13'W, approximately 2 m altitude). Further details on the meteorological event can be found at Regnet et al. (*in press*). The following specimens were examined: CHFURG 3477-3512, 3514-3543, 3546-3557, 3558-3560, 3590-3752, 3754-3877, 3896-3979, 4018-4041, 4080-4108, 4109-4140, 4324-4370, and 4640-4641.

Evaluation of population structure and morphometric parameters for Helicops infrataeniatus

For the determination of population parameters for *H. infrataeniatus*, each specimen was previously sexed through sub-caudal incision and verification of presence/absence of a hemipenis. Subsequently, measurements of total length (ToL), snout-vent length (SVL) and tail length (TaL) were taken with a flexible ruler. Individual weight was assessed with a digital scale to centesimal precision (0.01 g). Individuals were grouped into size classes using a step of 5 cm to construct histograms. Age groups within the *H. infrataeniatus* population were determined by the total length of each individual. Individuals with more than 400 mm ToL were considered adults, from 200 to 400 mm ToL were counted as juveniles, and with less than 200 mm were newborns (*sensu* Deiques and Cechin, 1991).

Lenght-weight relationships

The length-weight relationships (LWR) for females and males were calculated using the equation: $w = a \times L^b$, where W is the total weight of each snake (grams), *a* is a coefficient related to body shape, L is the total length (cm), and *b* is an exponent related to change in body shape (Froese, 2006). Parameters *a* and *b* were estimated by linear regression: $\log(W) = \log(a) + b\log(L)$. Outliers were excluded to provide a more accurate model (Froese, 2006).

Determination of sexual ratio for the population and sexual dimorphism

We applied a Yates corrected Chi-square test to determine if the studied population presents a sexual ratio of 1:1. Sexual dimorphism was based on external characters and evaluated through three variables: total length (ToL), tail length (TaL) and number of sub-caudal scales. For each variable, we tested the existence of significant differences between females and males using Mann-Whitney U Test. In this case we applied a Bonferroni Adjustment and considered significant values if $p < 0.025$.

Evaluation of ventral polichromatism

The ventral surface of each individual was photographed with a digital camera Canon PowerShot G10 (14.7 MP), in order to evaluate the frequency of occurrence of each ventral staining pattern type (morphotypes). We classified the found morphotypes according to terminology available in literature (*sensu* Boulenger, 1893; 1894; Giraudo, 2001; Lema, 2002) as follow: Morphotype A: trilinear staining pattern comprises specimens with three longitudinal black lines, between post-cephalic region and to the cloaca, with background color in shades of yellow or red. Morphotype B: checkered staining pattern includes specimens with absent longitudinal lines along the body, but with each ventral scale displaying alternating black coloration and background color in shades of yellow or red. Morphotype C: intermediate staining pattern includes specimens with both staining patterns described above on their ventral surface. To tested the existence of significant differences among ventral polichromatism, we used a Chi-square test.

Results

The analysis of 720 specimens of *H. infrataeniatus* showed the occurrence of 327 females (45.4%) and 376 males (52.2%) (sex ratio: 0.87:1; Yates corrected Chi-square $p = 0.54$). We were not able to identify the sex of 17 (2.4%) specimens. Twenty-nine specimens (13 females and 16 males) were excluded from the morphometric analysis due to their mutilated tails. The studied population is composed of 51.09% adult individuals, 47.59% juveniles, and 1.31% newborns. Meristic, morphometric and weight data for females and males of the 691 analyzed specimens are presented in Table 1.

The frequency distribution of individuals by size classes presented a bimodal pattern with more frequent sizes in the classes that include individuals between 20 and 25 cm (Figure 1). The most frequent ToL classes for females was between 19 cm (7.5%), while for males it was between 46 cm (6.7%).

The linear regression for LWRs analysis resulted on high determination coefficients for females and males ($r^2 = 0.98$; $r^2 = 0.97$, respectively, both $p < 0.001$). The intercept a was -3.22 for females and -2.93 for males, while estimates of parameter b was 2.85 and 2.60 for females and males respectively (see Figures 2 and 3).

The pholidosis of the sub-caudal scales in females ranged from 55 to 73 with a high frequency between 60 and 69 scales (84.84%), while in males ranged from 74 to 92 with values more frequent between 77 and 85 scales (87.22%). The most frequent numbers were 64 (11.93%) sub-caudal scales for females and 81 (14.16%) for males (Figure 4). Females had smaller TaL:ToL ratios than males. Mann-Whitney U Test showed highly significant differences between sexes for TaL:ToL ratios ($p < 0.001$) as well as the number of sub-caudal scales ($p < 0.001$).

Approximately 62% of all analyzed samples had ventral surface reddish patterns (31.4% females, 30% males and 0.6% without defined sex), while the others varied from shades of yellow (26%) to bicolor (red and yellow) (12%). The most common was the checkered pattern (38.4%), followed by the trilinear (35.8%) and the intermediate (25.6%) patterns (Figure 6). Both ventral staining pattern type presented highly significant values (χ^2 = 4467.000, p = 0.00; χ^2 = 3214.960, p = 0.00). No sexual dimorphism was observed regarding ventral polymorphism morphotypes.

One pregnant female revealed offspring composed by 24 fully formed embryos showing both types of ventral shades (yellowish and reddish). Ventral color patterns also varied among the embryos with 21 showing trilinear staining pattern, 2 checkered pattern, and one intermediate staining pattern. The embryos presented an average size of 18.5 cm. It was not possible to infer the sex of these individuals.

Discussion

Several aspects of *H. infrataeniatus* biology have been addressed in recent decades, including dietary data (Aguiar and Di-Bernardo, 2010), geographic distribution (Landgref-Filho and Aoki. 2011), ecology (Araujo and Ely, 1980), morphology (Santos-Costa and Hofstadler-Deiques, 2002), natural history (Lema, 1958a; Oliveira et al., 2004), reproduction (Aguiar and Di-Bernardo, 2005; Braz et al. 1988), and taxonomy (Lema, 1958b; Deiques and Cechin, 1991; Rossman, 2000). However, data regarding the population structure of this species were not available prior to the present study.

Our results provide strong evidence that *H. infrataeniatus* is very abundant in the study area as previously reported (Quintela and Loebmann, 2009; Braz, Scartozzoni and Almeida-Santos, 2016;) since all used specimens came from the same flood event as well as the same area (Regnet et al. *in press*).

Sexual dimorphism related to the total length of snakes has long been recognized (Blanchard, 1931). Adult females have larger body proportions than males (Shine, 1994) in species in which males do not present male-male combats or in viviparous and aquatic species. This happens because larger females, with larger body cavities, are able to generate larger offspring (Keogh, Branch and Shine 2000; Rivas and Burghardt, 2001; Ávila, Ferreira and Arruda, 2006). The fact that *H. infrataeniatus* is a viviparous and aquatic species, in combination with the lack of evidences of male-male combats, constitutes evidence that the pattern of sexual dimorphism found was expected.

Serpents are also dimorphic regarding caudal length, with males with larger tails and higher number of sub-caudal scales than females (Shine, 1984; Van Gelder et al. 1988). The number of sub-caudal scales in *H. infrataeniatus* can be used as an effective sexual dimorphic character, since there is no overlap in the number of this feature. Larger tails for males can mean an increase in reproductive success, providing more space to shelter a larger hemipenis (King, 1989; Shine et al. 1999). Accidental loss of tails or part of it, as was observed for 29 individuals, has been pointed as a factor that promotes decrease in male reproductive success (Shine et al. 1999). The reason that reproductive success may be influenced by tail mutilations is due to the fact that males with smaller tails have lower capacity to mate (King, 1999). Therefore, mutilation acts as a mechanism of sexual selection (King, 1999).

We verified the presence of the three morphotypes described in literature during analyzes of ventral polychromatism. Individual colorations and ventral staining patterns were unrelated to sex or age. Red coloration and checkered patterning were the predominant chromotypes in the studied population. Additionally, different patterns of ventral polymorphism may occur in the same offspring, as observed in one pregnant female and also previously reported by Quintela and Loebmann (2009). Polymorphism is reported for several species of snakes (e.g. Neill, 1963; Soini, 1974; Duarte, Freitas and Prudente, 2015) and according to some studies, this polymorphism may be linked to different variables such as behavior, thermoregulation, metabolism, interactions with the environment, prey-predator interactions, aposematism, camouflage and increased habitat occupation (Farallo and Forstner, 2012; Pizzatto and Dubey, 2012).

The vast majority of literature regarding the use of LWR for estimating species growth is related to fisheries resources, a research area in which this tool has been used for almost a century (e.g. Bertalanffy, 1934; Beverton and Holt, 1957). LWR parameters have been scarcely investigated in mammals (Cruz and Margarido, 2003), birds (Di-Campos et al. 2005), turtles (Lagler and Applegate, 1943; Dunson, 1967) lizards (Minnich, 1971; Meiri, 2010) or snakes (Kaufman and Gibbons, 1975; Feldman and Meiri, 2013). In this context, the present study presented novel LWR data based on an unprecedented large population sample, generating a solid basis for comparisons and future modelling.

The correlation between length and weight was high in the *H. infrataeniatus* analyzed samples, but still remained within the range reported for other species or groups of caenophidians (Kaufman and Gibbons, 1975; Feldman and Meiri, 2013). Slope values (parameter *b*) were similar to those found for a set composed by 166 species of colubrids and dipsadids (Feldman and Meiri, 2013). Intercept values (parameter *a*) were very distant from the values presented by Feldman and Meiri (2013) for other dipsadids as well as other species. When compared to intercepts of 13 species analyzed by Kaufman and Gibbons (1975), *a* values found for *H. infrataeniatus* were closer to the intercept of *Heterodon platyrhinos*. Slope values from the same work were similar to *H. platyrhinos* and *Agkistrodon piscivorous*, both in size and shape to *H. infrataeniatus*. Interestingly, Kaufman and Gibbons (1975) also found values of intercept very discrepant from the estimates of respective families presented by Feldman and Meiri (2013). Thus, equations based on single species with similar body size and shape may represent a better estimator than clade-specific equations at family level (Feldman and Meiri, 2013).

According to Fisher (1930), natural selection favors equal investment by females and males in the care of their offspring. Thus, it is expected that the number of females and males in a given population be similar. In fact, the majority of studies that have investigated population-level sex ratios in snakes corroborate the “Fisherian theory” (e.g. Shine and Bull, 1977; Ford, Brischoux and Lancaster, 2004; Pilgrim et al. 2011), as well as the studied population of *H. infrateniatus*. Ávila, Ferreira and Arruda (2006) found more males than females in a studied population of *H. leopardinus* while Ford and Ford (2002) found more females than males for *H. angulatus*, although in both cases they did not provide any statistical test.

The assessment of population structure is an important tool in the development of conservation strategies for any species. Freshwater and estuarine environments are facing an accelerated loss of biodiversity worldwide (Kennish, 2002; Vaughn, 2010). Therefore, data on population parameters represent extremely useful tools for future conservation plans.

Acknowledgements

R.A. Regnet thanks CAPES for Msc scholarships and C.N. de Paula, F. Caseiro, F.L. Silveira and V.H. Teixeira for the assistance in obtaining the morphological data of the specimens. F.M. Quintela is grateful to CAPES for postdoctoral fellowship.

Bibliography

Araujo, M.L., Ely, A.M. (1980): Incidência sazonal de *Helicops carinicaudus* (Jan, 1865) e *Philodryas patagoniensis* (Girard, 1857) no estado do Rio Grande do Sul, Brasil (Ophidia, Colubridae). Iheringia (série Zoologia) **55**: 87-106.

Aguiar, L.F.S., Di-Bernardo, M. (2005): Reproduction of the water snake *Helicops infrataeniatus* (Colubridae) in southern Brazil. Amphibia-Reptilia **26**: 527-533.

Aguiar, L.F.S., Di-Bernardo, M. (2010): Diet and feeding behavior of *Helicops infrataeniatus* (Serpentes: Colubridae: Xenodontinae) in Southern Brazil. Stud. Neotrop. Fauna Environ. **39**: 7-14.

Ávila R.W., Ferreira, V., Arruda, J.A.O. (2006): Natural history of the South American water snake *Helicops leopardinus* (Colubridae: Hydropsini) in the Pantanal, Central Brazil. J. Herpetol. **40**: 274-279.

Barnosky, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., Marshall, C., McGuire, J.L., Lindsey, E.L., Maguire, K.C., Ben Mersey, B., Ferrer, E.A. (2011): Has the Earth's sixth mass extinction already arrived? Nature **471**: 51-57.

Bertalanffy, L. von (1934): Untersuchungen über die Gesetzlichkeiten des Wachstums 1. Allgemeine Grundlangen der Theorie. Roux'Arch. Entwicklungsmech. Org. **131**: 613-653.

Beverton, R.J.H., Holt, S.J. (1957): On the dynamics of exploited fish populations, vol 19. Gt. Britain, Fishery Invest.

Blanchard, F.N. (1931): Secondary sex characters of certain snakes. Bull. Antivenin Inst. Am. **4**: 95-104.

Boulenger, G.A. (1893): Catalogue of the snakes in the British Museum - Natural History. vol 1. London.

Boulenger, G.A. (1894): Catalogue of the snakes in the British Museum - Natural History. vol 2. London.

Braz, H.B., Scartozzoni, R.R., Almeida-Santos, S.M. (2016): Reproductive modes of the South American water snakes: A study system for the evolution of viviparity in squamate reptiles. Zool. Anz. **263**: 33-44.

Costa, H.C., Santana, D.J., Leal, F., Koroiva, R., Garcia, P.C.A. (2016): A new species of *Helicops* (Serpentes: Dipsadidae: Hydropsini) from southeastern Brazil. Herpetologica **72**: 157-166.

Cruz, A.C.C., Margarido, T.C.C. (2003): Características reprodutivas de *Didelphis albiventris* Lund, 1840 (Mammalia-Marsupialia) na região metropolitana de Curitiba, Paraná, Brasil. Arq. Ciênc. Vet. Zool. UNIPAR **6**: 119-126.

Deiques, C.H., Cechin, S.Z. (1991): O status de *Helicops carinicaudus* (Wied, 1825) (Serpentes: Colubridae). Acta Biol. Leopold. **12**: 313-326.

Di-Campos, M.S., Carvalho, I.D., Braga-Filho, A.C., Vilela, R.A., Matos, M.S., Savastano Jr, H. (2005): Estimativa de correlações entre medidas morfométricas, peso do ovo e peso de filhotes de emas criadas em cativeiro. Cienc. Rural **35**: 678-683.

Duarte, M.O., Freitas, T.M.S., Prudente, A.L.C. (2015): Polychromatism of populations of *Corallus hortulanus* (Squamata: Boidae) from the southern Amazon Basin, Brazil. Acta Amaz. **45**: 373-382.

Dunson, W.A. (1967): Relationship between length and weight in the spiny soft-shell turtle. Copeia **1967**: 483-485.

Farallo, V.R., Forstner, M.R.J. (2012): Predation and the maintenance of color polymorphism in a habitat specialist squamate. PLoS One **7**: 1-11.

Feldman, A., Meiri, S. (2013): Length-mass allometry in snakes. Biol. J. Linnean. Soc. **108**: 161-172.

Fisher, R.A. (1930): The genetical theory of natural selection. Clarendon Press, Oxford.

Fitch, H.S. (1975): A demographic study of the ringneck snake (*Diadophis punctatus*) in Kansas. University of Kansas Museum of Natural History. Misc. Publ. **62**: 1-53.

Ford, N.B., Ford, D.F. (2002): Notes on the ecology of the South American water snake *Helicops angulatus* (Squamata: Colubridae) in Nariva Swamp, Trinidad. Caribb. J. Sci. **38**: 129-132.

Ford, N.B., Brischoux, F., Lancaster, D. (2004): Reproduction in the western cottonmouth, *Agkistrodon piscivorus leucostoma*, in a floodplain forest. Southwestern Naturalist **49**: 465-471.

Fouquet, A., Loebmann, D, Castroviejo-Fisher, S., Padias, J. M., Orrico, V.G.D., Lyra, M.L., Roberto, I.J., Kok, P.J.R., Haddad, C.F.B., Rodrigues, M.T. (2012): From Amazonia to the Atlantic Forest: molecular phylogeny of Phyzelaphryninae frogs reveals unexpected diversity and a striking biogeographic pattern emphasizing conservation challenges. Mol. Phylogenet. Evol. **65**: 547-561.

Froese, R. (2006): Cube law, condition factor and weight-length relationships: History, meta-analysis and recommendations. J. Appl. Ichthyology **22**: 241-253.

Giraudo, A. (2001): Serpientes de la Selva Paranaense y del Chaco Húmedo. Buenos Aires, Literature of Latin America.

Horreo, J.L., Fitze, P. (2015): Population structure of three *Psammodromus* species in the Iberian Peninsula. PeerJ. **3**: e994.

IUCN (2012): IUCN Red List Categories and Criteria: Version 3.1. 2nd Edition. Gland, Switzerland and Cambridge, UK.

Jackson, J.B.C. (2008): Ecological extinction and evolution in the brave new ocean. Proc. Natl. Acad. Sci. **105**: 11458-11465.

Kaufman, G.A., Gibbons, J.W. (1975): Weight-length relationships in thirteen species of snakes in the Southeastern United States. Herpetologica **31**: 31-37.

Kennish, M.J. (2002): Environmental threats and environmental future of estuaries. Environ. Conserv. **29**: 78-107.

Keogh, J.S., Branch, W.R., Shine, R. (2000): Feeding ecology, reproduction and sexual dimorphism in the colubrid snake *Crotaphopeltis hotamboeia* in southern Africa. Afr. J. Herpetol. **49**: 129-137.

King, R.B. (1989): Sexual dimorphism in snake tail length: sexual selection, natural selection, or morphological constraint? Biol. J. Linn. Soc. **38**: 133-154.

Lagler, K.F., Applegate, V.C. (1943): Relationship between length and the weight in the snapping turtle, *Chelydra serpentina* Linnaeus. Am. Nat. **77**: 476-478.

Landgref-Filho, P., Aoki, C. (2011): *Helicops infrataeniatus* (water snake). Brazil: Mato Grosso do Sul. Herpetol. Rev. **42**: 394.

Lema, T.L. (1958a): Nota prévia sobre a espécie de colubrídeo aquático *Helicops carinicauda* (Wied, 1825) do estado do Rio Grande do Sul, Brasil. Notas sobre os répteis do estado do Rio Grande do Sul - Brasil. Iheringia Sér. Zool. **10**: 21-24.

Lema, T.L. (1958b): Bicefalia em *Helicops carinicauda* (Wied). Descrição de um caso. Notas sobre os répteis do estado do Rio Grande do Sul - Brasil. Iheringia Sér. Zool. **10**: 25-27.

Lema, T.L. (2002): Os répteis do Rio Grande do Sul: atuais e fósseis - biogeografia - ofidismo. Edipucrs.

Meiri, S. (2010): Length-weight allometries in lizards. J. Zool. **281**: 218-226.

Millar, C.I., Libby, W.J. (1991): Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species. New York: Oxford University Press.

Minnich, J.E. (1971): Seasonal variation in weight-length relationships and fat body size in the desert iguana, *Dipsosaurus dorsalis*. Copeia **1971**: 359-362.

Neill, W.T. (1963): Polychromatism in snakes. Quart. J. Florida Acad. Sci. **26**: 194-216.

Oliveira, R.B., Pontes, G.M.F., Solé, M., Di-Bernardo, M., Borges-Martins, M. (2004): *Lystrophis dorbignyi* (nariguda) e *Helicops infrataeniatus* (cobra d'água). Predation. Herpetologial Reviev **35**:70.

Pilgrim, M.A. Farrell, T.M. May, P.G., Vollman, M.R. Siegel, R.A. (2011). Secondary sex ratios in six snake species. Copeia **4**: 553-558.

Pizzatto, L., Dubey, S. (2012): Colour-polymorphic snake species are older. Biol. J. Linnean Soc. **107**: 210-218.

Quintela, F.M., Loebmann, D. (2009): Guia ilustrado - Os répteis da região costeira do extremo sul do Brasil. Pelotas, RS. Useb.

Regnet, R.A, Quintela, F.M., Entiauspe-Neto, O.M., Teixeira, V.H., Silveira, F.L., Loebmann, D. (*in press*): Heavy rainfall associated to *El Niño* promotes cyclical aggregation of amphibians and reptiles in Southern Brazil. Salamandra.

Rivas, J.A., Burghardt, G.M. (2001): Understanding sexual dimorphism in snakes: wearing the snake's shoes. Anim. Behav. **62**: F1-F6.

Robinson, J.G. (2006): Conservation biology and real-world conservation. Conserv. Biol. **20**: 658-660.

Rocha, C.F.D. (1998): Population dynamics of the endemic tropidurid lizars *Liolaemus lutzae* e seazonal restinga habitat. Cienc. Cult. **50**: 446-451.

Rossman, D. A. (2000): Identity of the snake *Calopisma septemvittatum* FISCHER, 1879, and redescription of the lectotype of *Helicops infrataeniatus* JAN, 1865. Mitteilungen aus dem Zoologischen Museum in Berlin **97**: 123-129.

Santos-Costa, M.C., Hofstadler-Deiques, C. (2002): The ethmoidal region and cranial adaptations of the neotropical aquatic snake *Helicops infrataeniatus* Jan, 1865 (Serpentes, Colubridae). Amphibia-Reptilia **23**: 83-91.

Shine, R. (1984): Reproductive biology and food habits of the Australian elapid snakes of the genus *Cryptophis*. J. Herpetol. **18**: 33-39.

Shine, R., Bull. J. J. (1977): Skewed sex ratios in snakes. Copeia **1977**: 228-234.

Shine, R. (1994): Sexual size dimorphism in snakes revisited. Copeia **1994**: 326-346.

Shine, R., Olsson, M.M., Moore, L.T., LeMaster, M.P., Mason, R.T. (1999): Why do male snakes have longer tails than females? Proc. R. Soc. Lond. B. **266**: 2147-2151.

Soini, P. (1974): Polychromatism in a population of *Micrurus langsdorffi*. J. Herpetol. **8**: 267-269.

Sun, L-X., Shine, R., Debi, Z., Zhengren, T. (2001): Biotic and abiotic influences on activity patterns of insular pit-vipers (*Gloydius shedaoensis*, Viperidae) from north-eastern China. Biol. Cons. **97**: 387-398.

Sutherland, W.J., Adams, W.M., Aronson, R.B., Aveling, R., Blackburn, T.M., Broad, S., Ceballos, G., Cote, I.M., Cowling, R.M., Da Fonseca, G.A.B., Dinerstein, E., Ferraro, P.J., Fleishman, E., Gascon, C., Hunter Jr., M., Hutton, J., Kareiva, P., Kuria, A., Macdonald, D.W., Mackinnon, K., Madgwick, F.J., Mascia, M.B., Mcneely, J., Milner-Gulland, E.J., Moon, S., Morley, C.G., Nelson, S., Osborn, D., Pai, M., Parsons, E.C.M., Peck, L.S., Possingham, H., Prior, S.V., Pullin, A.S., Rands, M.R.W., Ranganathan, J., Redford, K.H., Rodriguez, J.P., Seymour, F., Sobel, J., Sodhi, N.S., Stott, A., Vance-Borland, K., Watkinson, A.R. (2009): One hundred questions of importance to the conservation of global biological diversity. Conserv. Biol. **23**: 557-567.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., Siqueira, M.F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., Jaarsveld, A.S. van., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L., Williams, S.E. (2004): Extinction risk from climate change. Nature **427**: 145-148.

Uller, T. (2003): Viviparity as a constraint on sex-ratio evolution. Evolution **57:** 927-931.

Van Gelder, J.J., Olders, J.H.J., Mertens, L.A.J.M., Kersten, H.L.M. (1988): Field identification of the sex of the smooth snake (*Coronella austriaca* Laurenti). J. Herpetol. **22**: 53-63.

Vaughn, C.C. (2010): Biodiversity losses and ecosystem function in freshwaters: emerging conclusions and research directions. BioScience **60**: 25-35.

Table and figures

Table 1. Meristic, morphometric and weight for females and males data from the 691 specimens of *H. infrataeniatus*. Results expressed in mean, standard deviation and amplitude. Abbreviations SVL = Snout-vent length, ToL = Total Length, TaL = Tail Length, SUB = Number of subcaudal scales.

N	SVL (cm)	ToL (cm)	TaL (cm)	SVL/TaL	SUB	Weight (g)
319	31.42±13.32	41.36±17.1	9.9±4.05	0.25±0.02	64.7±3.53	37.65±40.35
(Females)	13.3-62.5	18.4-78.2	4-18.5	0.02-0.3	55-73	2.18-191.02
372	26.49±8.68	37.8±12.81	11.46±4.32	0.3±0.22	81.1±2.99	18.58±14.36
(Males)	13.9-46.7	19.7-67	4.9-21	0.22-0.47	74-92	2.30-61.27

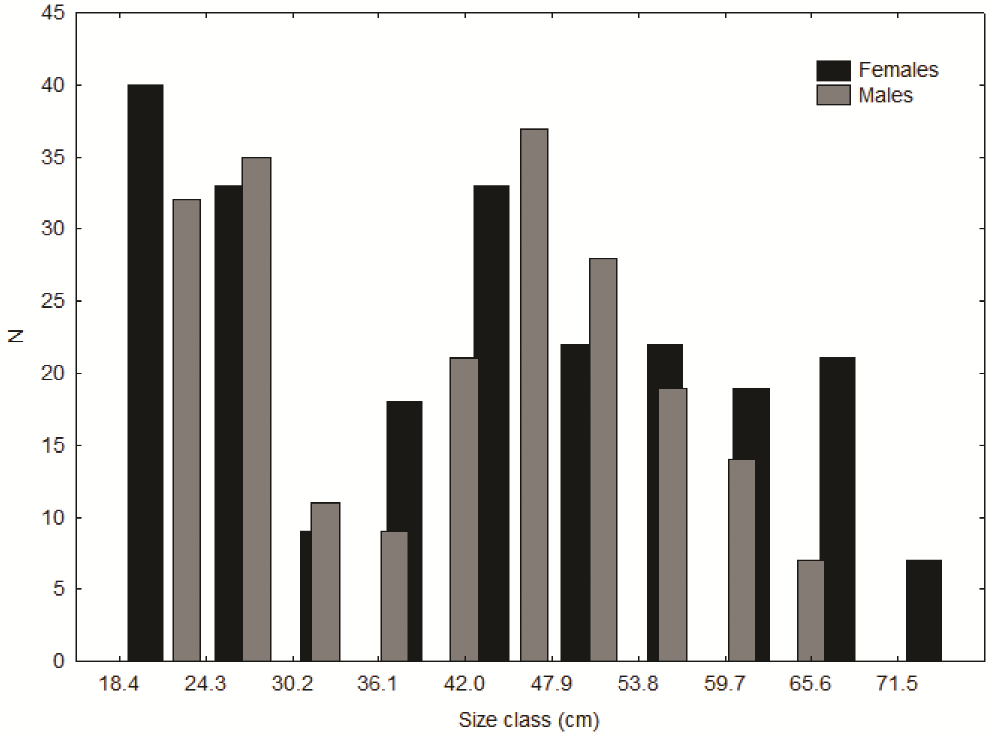

Figure 1. Frequency distributions of size classes for specimens of *Helicops infrataeniatus* from the Praia do Laranjal, municipality of Pelotas, state of Rio Grande do Sul, Brazil.

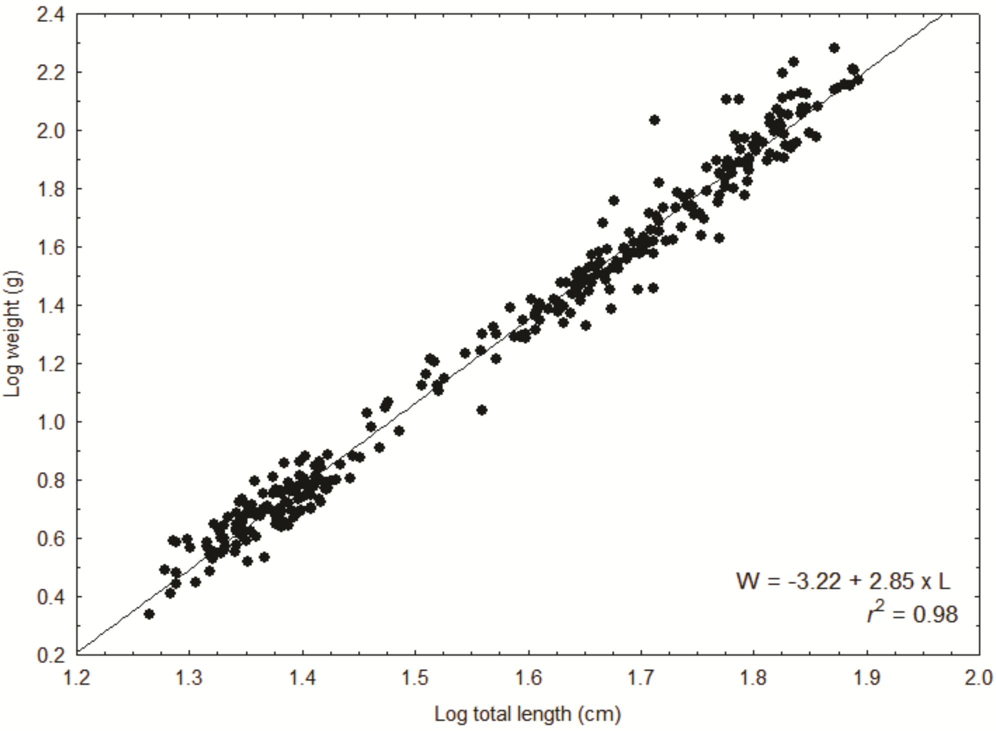

Figure 2. Length-weight relationships for females of *H. infrataeniatus*.

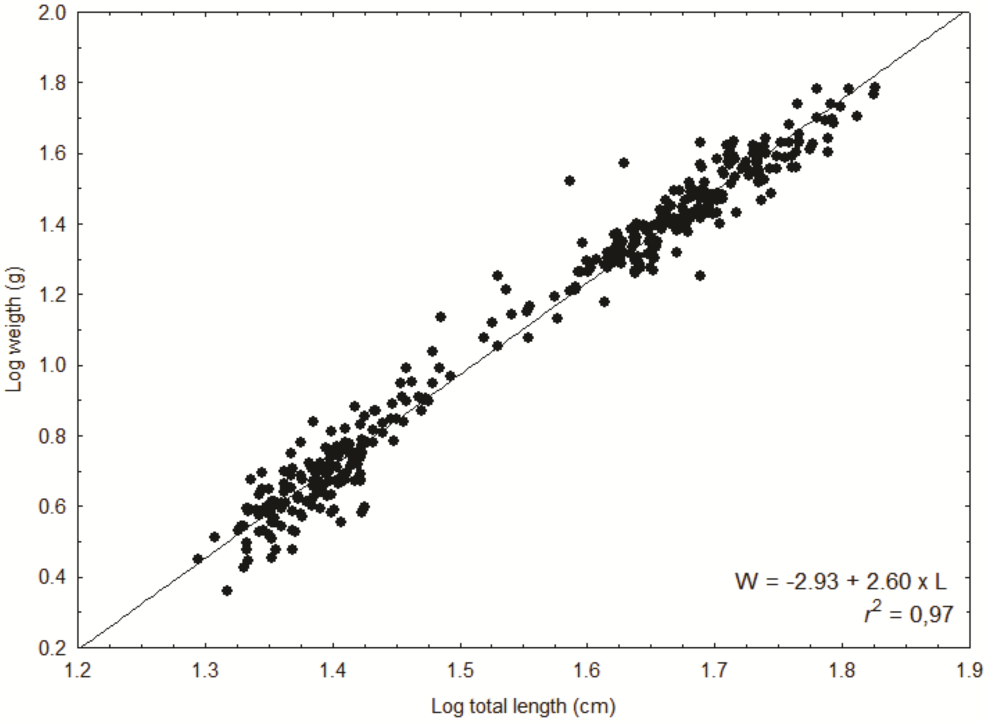

Figure 3. Length-weight relationships for males of *H. infrataeniatus*.

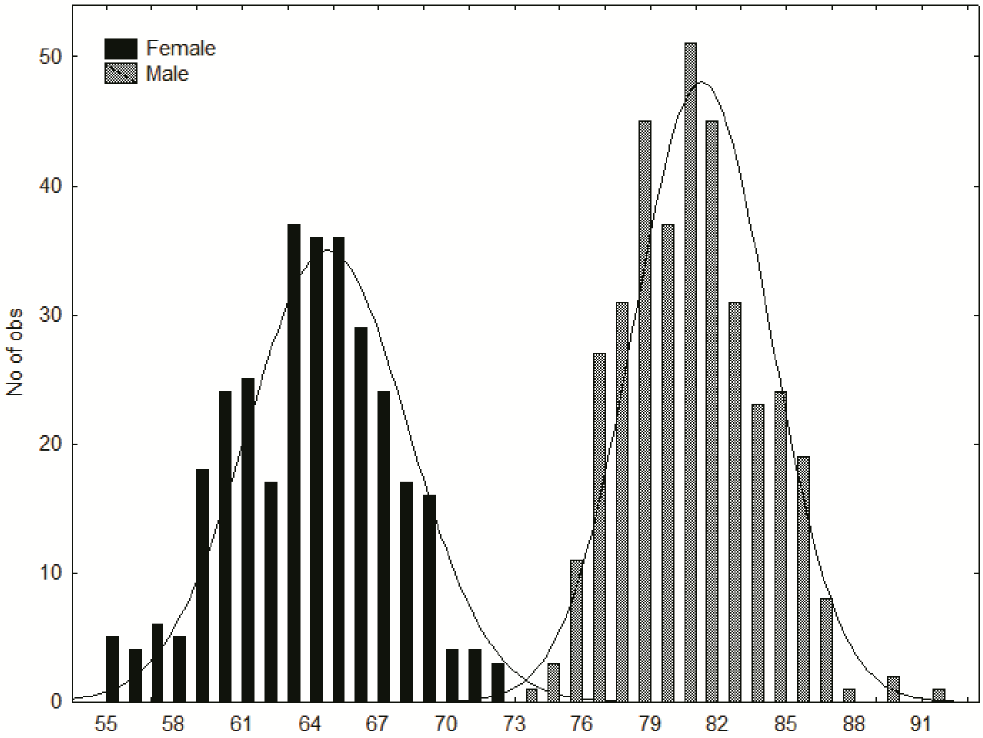

Figure 4. Histograms showing the frenquency of occurrence of the number of subcaudal scales for females and males of *H. infrataeniatus*.

Figure 6. Ventral surface of *Helicops infrataeniatus* and their respective morphotypes and color variations. A - intermediate staining patterns; B - trilinear staining patterns and C - checkered staining patterns.

CAPÍTULO 3

Manuscrito submetido para The Herpetological Journal - The British Herpetological Society.
Normas de formatação do periódico encontram-se no anexo quatro desta dissertação.

Título: Intersexuality in *Helicops infrataeniatus* Jan, 1883 (Dipsadidae: Hydropsini) causes abnormalities in feminine and masculine reproductive organs

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Número de palavras: 2.093

Figuras: 1

Qualis Capes 2017: B2

Fator de impacto: 0,90

Intersexuality in *Helicops infrataeniatus* Jan, 1883 (Dipsadidae: Hydropsini) causes abnormalities in feminine and masculine reproductive organs

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Running title: Intersexuality in *Helicops infrataeniatus*

Key words: Follicles, hemipenis, hermaphroditism, water snake.

Abstract Herein, we describe the first case of intersexuality in the Hydropsini tribe. After examination of 720 specimens of *Helicops infrataeniatus* Jan, 1883, we have discovered one individual that presented feminine and masculine reproductive features. The specimen was 619 mm long, with seven follicles in secondary stage, of different shapes and sizes, and hemipenis with 13.32 and 13.57 mm in length. General shape of this organ is similar to that observed in males, although it is smaller and does not present conspicuous spines along its body. Deformities found in feminine and masculine structures suggest that this specimen might not be reproductively functional.

As all other amniotes non-volant, reptiles have internal fecundation and, with the exception of Tuataras, which have no copulatory organ, male specimens have hemipenises (Vitt & Caldwell, 2013). However, some reptile species evolved to break free of sexual reproduction, being the only vertebrates that truly reproduce by parthenogenesis (Kearney et al., 2009; Vitt & Caldwell, 2013). Such mechanism is predominant in lizards (e.g. Darevski, 1966; Hardy & Cole, 1981; Schmidtler, 1993; Schmidtler et al., 1994), but it also occurs in snakes (e.g. Wynn et al., 1987; Groot et al., 2003; Booth et al., 2012). Another condition reported for reptiles is intersexuality, which is defined as a condition in which reproductive structures of a given sex are also found in the opposite sex (Goldschmidt, 1915). The condition may include both hermaphroditism (presence of both ovarian and testicular tissues) and pseudohermaphroditism (presence of gonadal tissue of one single sex) (Forbes, 1964).

In squamate reptiles, cases of intersexuality have been reported for lizards (e.g. Böhme, 1995; Ziegler & Böhme, 1997; Ziegler et al., 1999; Telemeco, 2015; Valdecantos & Lobo, 2015), amphisbaenians (e.g. Ziegler & Böhme, 1997; Kasperoviczus et al., 2011) and snakes (e.g. Hoge et al., 1954; Hoge & Penha, 1959; Maclean, 1968; Hardy, 1970; Ziegler & Böhme, 1997). The intersexual condition in these groups is usually evidenced by the presence of erectile organs in females similar to the male hemipenis (Forbes, 1964). Despite the similarity, some structures such as the *ductus spermaticus* and spines may be underdeveloped (Hardy, 1970; Kasperoviczus et al., 2011). Among snakes, the presence of a hemiclitoris has already been verified in *Pareas stanleyi* (Pope, 1935), *Bothrops insularis* (Hoge et al., 1954; Hoge & Penha, 1959), *B. moojeni* (Maclean, 1968), *Pseudoficimia frontalis* (Hardy, 1970), and *Natrix natrix* (Ziegler & Böhme, 1997).

The Hydropsini tribe currently encompasses a total of 22 valid species allocated in the following genera: *Helicops*, *Hydrops* and *Pseudoeryx* (Uetz et al., 2017). While *Hydrops* and

Pseudoeryx are oviparous, most species of *Helicops* are viviparous, and interestingly, *H. angulatus* exhibits both reproductive modes (Rossman, 1984; Aguiar & Di-Bernardo, 2005, Braz et al., 2016). *Helicops infrataeniatus* is a common species in many types of limnic and estuarine environments in the coastal zone of southernmost Brazil (Quintela & Loebmann, 2009, Regnet et al., *in press*). In October 2015, a remarkable aggregation of reptiles and caecilians occurred after a flood event associated to an El Niño event (Regnet et al., *in press*), extraordinarily making possible to investigate the population structure and aspects of the reproductive biology of several species with a large sample size. When such specimens were examined, one showed follicles in advanced vitellogenesis as well as hemipenis, characterizing a rare case of intersexuality, which is here described.

The intersexual specimen was collected at the Laranjal beach, municipality of Pelotas, state of Rio Grande do Sul (31°46'S, 52°13'W) and deposited in the Herpetological Collection of the Universidade Federal do Rio Grande - FURG (CHFURG 3946). Although the individual presented female and male sexual characteristics, it was considered female, once the number of sub-caudal scales (64) was too low to classify as male, which have at least 74 scales (Giraudo 2001; Regnet et al., unpub. data). For comparison purposes, we everted and inflated a total of four males hemipenes as well as follicles in secondary vitellogenesis from a female. For each specimen studied, we measured the hemipenis length as well as snout-vent length (SVL). For standardization, we used only the length of the right hemipenis. Description of reproductive organs follows Zaher (1999) for hemipenis and Almeida-Santos et al. (2014), for follicles.

Snout-vent length of the intersexual specimen (Fig. 1) measured was 537 mm, tail length was 82 mm and weight was 103.87 g. The left ovary contained seven follicles in secondary stage, with different shapes and sizes, and follicles of the right ovary were all in the primary stage (Fig. 1). Right and left hemipenis were 13.32 and 13.57 mm long, respectively.

Males had SVL ranging from 380 to 432 (mean = 410.5 mm) and right hemipenis length ranging from 13.79 to 14.61 (mean = 14.17 mm). Relative measures (organ length/SVL) of right hemipenis were 0.025 and 0.035 on average, respectively. General shape of hemipenis in both sides (assulcate and sulcate) is similar in both structures, although the hemipenis of the intersexual is smaller (ca. 71.43% in relation to the mean length of hemipenises measured). Both structures are bilobated, but only hemipenes have conspicuous spines along their body (Fig. 1).

The intersexual specimen here described was detected in a large sample of 720 individuals (327 females and 376 males), that is, it represents only 0.14% of the studied population. Therefore, the condition should be considered rare for this species. Aguiar & Di-Bernardo (2005) provide biological data on the reproduction of *H. infrataeniatus* and, when comparing those data to the observation of the intersexual specimen, the number of follicles found is to be expected. However, follicles from the intersexual specimen were highly variable in size, shape and color as well, so it is possible that intersexual follicles may not be viable. This condition differs from previous studies, which described individuals with hemiclitoris in *Bothrops insularis* (Hoge et al., 1954), *Bothrops moojeni* (Mcclean, 1968), *Pseudoficimia frontalis* (Hardy, 1970), and *Pareas stanleyi* (Pope, 1935), in which all of them were fertile females. Additionally, *B. insularis* has high incidence of females with hemiclitores and, surprisingly, individuals without the structure seem to be sterile (Hoge et al., 1959).

As expected, the hemipenis of the intersexual specimen was smaller and more translucent in relation to a male hemipenis. Besides this, intersexual specimen did not present seminiferous tubules or testicles.

This is the first report of an intersexual individual in the Hydropsini tribe. The presence of intersexual individuals emphasizes the importance to check other sexual features concomitantly (Ziegler & Böhme, 1996), in order to avoid erroneous sex determination. An interesting case can be found in the description of *Pseudoficimia pulcherrima*, in which two intersexual females where used to describe the male hemipenis (Taylor & Smith, 1942). After the mistake was discovered, the species was simonimized with *Pseudoficimia frontalis* (Hardy, 1970). We concluded that sexual determination based on the presence of a structure similar to a hemipenis might not be conclusive and, therefore, this issue needs to be evaluated more criteriously to avoid possible bias in sexual determination.

Acknowledgements

R.A. Regnet thanks CAPES for the Msc. scholarship and F.M. Quintela is grateful to CAPES for the postdoctoral fellowship.

References

Aguiar, L. F. S. & Di-Bernardo, M. (2005). Reproduction of the water snake *Helicops infrataeniatus* (Colubridae) in southern Brazil. *Amphibia-Reptilia* **26**, 527-533.

Almeida-Santos, S. M., Braz, H. B. P., Santos, L. C., Barros, V. A., Rojas, C. A. & Kasperoviczus, K. N. (2014). Biologia reprodutiva de serpentes: recomendações para a coleta e análise de dados. *Herpetologia Brasileira* **3**, 14-24.

Böhme, W. (1995). Hemiclitoris discovered: a fully differentiated erectile structure in female monitor lizards (*Varanus* spp.) (Reptilia: Varanidae). *Journal of Zoological Systematics and Evolutionary Research* **33**, 129-132.

Booth, W., Smith, C. F., Eskridge, P. H., Hoss, S. K., Mendelson, J. R. & Schuett, G. W. (2012). Facultative parthenogenesis discovered in wild vertebrates. *Biology letters* **8**, 983-985.

Braz, H. B., Scartozzoni, R. R. & Almeida-Santos, S. M. (2016). Reproductive modes of the South American water snakes: A study system for the evolution of viviparity in squamate reptiles. *Zoologischer Anzeiger* **263**, 33-44.

Darevski, I. S. (1966). Natural Parthenogenesis in a Polymorphic Group of Caucasian Rock Lizards Related to *Lacerta saxicola* Eversmann. *Journal of the Herpetological Society* **5**, 115-152.

Forbes, T. R. (1964). Intersexuality in reptiles. In *Intersexuality in vertebrates including man*, 273-284. Armstrong, C. N. & Marshall, A. J. (Eds.). Academic Press, New York.

Giraudo, A. R. (2001). *Serpientes de la Selva Paranaense y del Chaco Húmedo*. Literature of Latin America, Buenos Aires, Argentina, 328p.

Groot, T. V. M., Bruins, E. & Breeuwer, J. A. J. (2003). Molecular genetic evidence for parthenogenesis in the *Burmese python*, *Python molurus bivittatus*. *Heredity* **90**, 130-135.

Goldschmidt, R. (1917). Intersexuality and the endocrine aspect of sex. *From the Osborn Zoological Laboratory, Yale University* **1**, 433-456.

Hardy, L. M. (1970). Intersexuality in a Mexican Colubrid Snake (*Pseudoficimia*). *Herpetologica* **26**, 336-343.

Hardy, L. M. & Cole, C. J. (1981). Parthenogenetic reproduction in lizards: histological evidence. *Journal of Morphology* **170**, 215-237.

Hoge, A., Belluomini, H. & Schreiber, G. (1954). Intersexuality in a highly isolated population of snakes. Atti *del IX Congresso Internazionale di Genetica, Caryologica, Vol. suppl. p.* 964-965.

Hoge, A. & Penha, A. M. (1959). Sexual abnormalities in *Bothrops insularis* (Amaral) 1921. *Memórias do Instituto Butantan* **29**,17-88.

Kaspervoviczus, K. N., Santos, L. C. & Almeida-Santos, S. (2011). First report of hemiclitores in a female of the amphisbaenian *Amphisbaena microcephala* (Wagler, 1824). *Herpetology notes* **4**, 41-43.

Kearney, M., Fujita, M. K., & Ridenour, J. (2009). Lost Sex in the Reptiles: Constraints and correlations. In *Lost Sex - The Evolutionary Biology of Parthenogenesis,* 447-474. Schön, I., Martens, K. & van Dijk, P. (Eds.). Springer, Dordrecht, Heidelberg, London, New York.

Maclean, W. P. (1968). A case of intersexuality in *Bothrops moojeni* Hoge. *Copeia* **1968**, 170.

Pope, C. H. (1935). *The reptiles of China.* American Museum of Natural History. 604 p.

Quintela, F.M. & Loebmann, D. (2009). *Guia Ilustrado: os répteis da região costeira do Rio Grande do Sul.* Pelotas, USEB, 84 p.

Rossman, D. A. (1984). *Helicops angulatus* (South American Water Snake). Reproduction. *Herpetological Review* **15**: 50.

Regnet, R. A., Quintela, F. M., Entiauspe-Neto, O. M., Teixeira, V. H. S., Silva, F. L., Loebmann, D. *in press*. Remarkable aggregation of squamates and caecilians associated with flood events during *El Niño* in southern Brazil.

Schmidtler, J. F. (1993). Parthenogenetische Felseideshsen aus de Ost-Türkei. *Die Eidechse.* **8**: 3-5.

Schmidtler, J. F., Eiselt, J. & Darevsky, I. S. (1994). Untersuchungen an Felseidechsen (Lacerta saxkola-Gruppe) in der östlichen Türkei: 3. Zwei neue parthenogenetische Arten. *Salamandra* **30**, 55-70.

Telemeco, R. S. (2015). Sex Determination in Southern Alligator Lizards (*Elgaria multicarinata*; Anguidae). *Herpetologica* **71**, 8-11.

Uetz, P., Freed, P. & Hošek, J. (2017) The Reptile Database, http://www.reptile-database.org, accessed June 5th.

Valdecantos, S. & Lobo, F. (2015). First Report of Hemiclitores in Females of South American Liolaemid Lizards. *Journal of Herpetology* **49**, 291-294.

Vitt, L. J. & Caldwell, J. P. (2013). *Herpetology: an introductory biology of amphibians and reptiles*. Academic Press.

Ziegler, T. & Böhme, W. (1996). Zur Hemiclitoris der squamaten Reptilien: Auswirkingen auf einige Methoden der Geschlechtsunterscheidung. Herpetofauna (Weinstadt) **18**, 11-19.

Ziegler, T. & Böhme, W. (1997). Genitalstrukturen und Paarungsbiologie bei squamaten Reptilien, speziell den Platynota, mit Bemerkungen zur Systematik. *Mertensiella, Supplement zu Salamandra* **8**, 1-207.

Ziegler, T., Böhme, W. & Philipp, K. M. (1999). *Varanus caerulivirens* sp. n., a new monitor lizard of the *V. indicus* group from Halmahera, Moluccas, Indonesia (Squamata: Sauria: Varanidae). *Herpetozoa* **12**, 45-56.

Zaher, H. (1999). Hemipenial morphology of the South American xenodontine snakes, with a proposal for a monophyletic Xenodontinae and a reappraisal of colubroid hemipenes. *Bulletin of the American Museum of Natural History* **240**, 1-168.

Wynn, A., Charles, H., Cole, J. & Gardner. A. L. (1987). Apparent triploidy in the unisexual brahminy blind snake, *Ramphotyphlops braminus*. *American Museum Novitates* **2868**, 1-7.

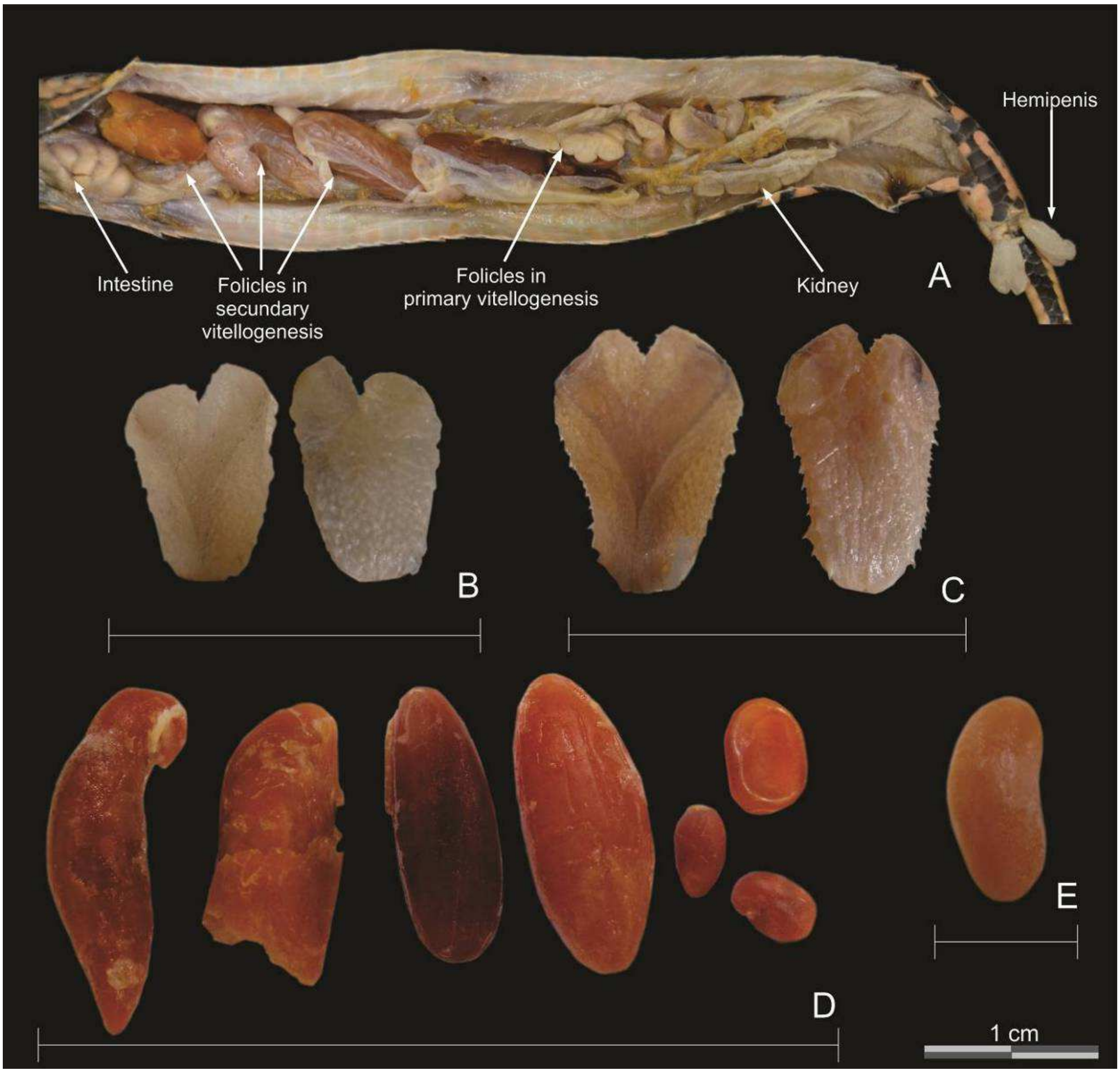


Figure 1. A - General view of reproductive organs of the intersexual specimen of *Helicops infrataeniatus*; B – intersexual hemipenis; C – male hemipenis; D – follicles of the intersexual specimen with different shapes and sizes and E - follicle of a normal female.

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

O evento de agregação de milhares de indivíduos de anfíbios e répteis, reportado e discutido no primeiro capítulo desta dissertação, nos proporcionou a oportunidade de discutir sobre os impactos de eventos de inundação na região estuarina da Lagoa dos Patos, extremo sul do Brazil na herpetofauna. Os resultados obtidos neste capítulo demonstram que as inundações, principalmente associadas ao El Niño, influenciam diretamente a herpetofauna que habita ambientes adjacentes à região estuarina. Acreditamos que os indivíduos amostrados na agregação tenham origem nos banhados da várzea do canal de São Gonçalo, uma vez que não foram encontrados espécimes nas praias mais ao norte da Praia do Laranjal. No entanto, é fundamental que outros eventos similares sejam monitorados para gerar dados mais detalhados, identificando a origem destes indivíduos e como estes eventos impactam a herpetofauna.

A partir de uma amostra representativa da cobra-d'água *Helicops infrataeniatus,* obtida durante o evento relatado no primeiro capítulo, geramos dados populacionais ainda desconhecidos acerca da biologia desta espécie. Apresentamos ainda resultados inéditos de estrutura populacional, proporção e dimorfismo sexual, relações peso-comprimento de machos e fêmeas, além da quantificação da variação de colorido ventral. Essa base de dados pode ser replicada para outras espécies com número representativo em coleções científicas. Isso demonstra a importância da coleta contínua de espécies e o cuidado que deve-se ter ao depositá-los em coleções, permitindo assim que lacunas do conhecimento sejam gradativamente preenchidas.

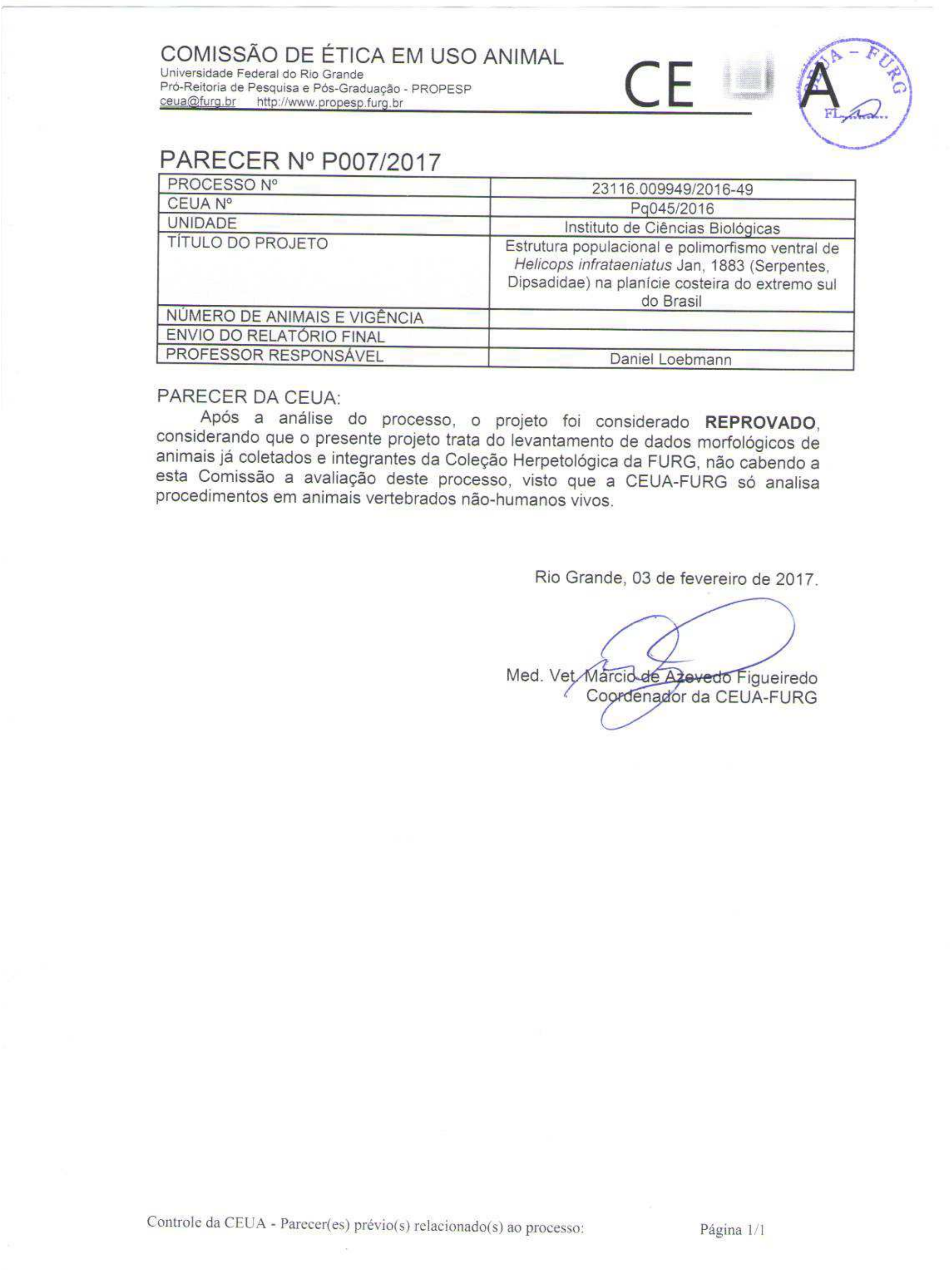
Além disso, os dados obtidos no segundo capítulo são uma ferramenta importante no desenvolvimento de estratégias de conservação para *H. infrataeniatus*, pois representa uma base de dados até então inexistente para a espécie. Isto é particularmente importante pelo fato de esta ser uma espécie tipicamente aquática, ambiente considerado um dos mais afetados em relação à perda de diversidade global.

Relatamos ainda o primeiro caso de intersexualidade para a tribo Hydropsini, que foi descoberto após o exame minucioso da população de *H. infrataeniatus* estudada. Discutimos a importância da determinação sexual mais acurada com base em outras características sexuais, uma vez que a presença de hemipenis tem sido utilizado como uns dos principais caracteres e muitas vezes o único, na determinação sexual em répteis.

Durante o evento de inundação relatado e discutido, foram coletados espécimes de répteis e anfíbios. Todos os espécimes de répteis foram estudados nesta dissertação, inclusive tendo sido descrita uma nova espécie de cobra-de-vidro *Ophiodes* sp. junto à agregação. No entanto, é importante ressaltar que o mesmo evento permitiu a coleta de dezenas de espécimes de *Chthonerpeton indistinctum*, um dos elementos da herpetofauna mais difíceis de serem amostrados na região, devido ao seu hábito fossorial. Portanto, espera-se que esses espécimes que estão depositados na Coleção Herpetologia da Furg sejam analisados em um futuro próximo.

ANEXO 1

Parecer da Comissão de Ética em Uso Animal da Universidade Federal do Rio Grande.



COMISSÃO DE ÉTICA EM USO ANIMAL
Universidade Federal do Rio Grande
Pró-Reitoria de Pesquisa e Pós-Graduação - PROPESP
ceua@furg.br http://www.propesp.furg.br

PARECER Nº P007/2017

PROCESSO Nº	23116.009949/2016-49
CEUA Nº	Pq045/2016
UNIDADE	Instituto de Ciências Biológicas
TÍTULO DO PROJETO	Estrutura populacional e polimorfismo ventral de *Helicops infrataeniatus* Jan, 1883 (Serpentes, Dipsadidae) na planície costeira do extremo sul do Brasil
NÚMERO DE ANIMAIS E VIGÊNCIA	
ENVIO DO RELATÓRIO FINAL	
PROFESSOR RESPONSÁVEL	Daniel Loebmann

PARECER DA CEUA:

Após a análise do processo, o projeto foi considerado **REPROVADO**, considerando que o presente projeto trata do levantamento de dados morfológicos de animais já coletados e integrantes da Coleção Herpetológica da FURG, não cabendo a esta Comissão a avaliação deste processo, visto que a CEUA-FURG só analisa procedimentos em animais vertebrados não-humanos vivos.

Rio Grande, 03 de fevereiro de 2017.

Med. Vet. Márcio de Azevedo Figueiredo
Coordenador da CEUA-FURG

Controle da CEUA - Parecer(es) prévio(s) relacionado(s) ao processo: Página 1/1

ANEXO 2

Instructions for authors - Salamandra German Journal of Herpetology

Scope SALAMANDRA is a broadly based herpetological journal of the Deutsche Gesellschaft für Herpetologie und Terrarienkunde e.V. (DGHT). It publishes results of original research and review articles in all fields of herpetology, including phylogeny, systematics, ethology, ecology, physiology, conservation biology, and captive breeding, given a respective scientific relevance. Shorter contributions on faunistics and natural history, as well as species checklists are only accepted, if findings provide substantive new discernments.

Submission Manuscripts have to be submitted electronically to DGHT: salamandra@dght.de

Articles are exclusively published in English (preferably British English). If you are not a native speaker, it is recommended to consult a native speaker to review your manuscript prior to submission.

Authors must suggest at least two potential referees accompanied by their e-mail addresses. These suggested persons should not be close collaborators and should not have participated in joint publications recently.

Submitted manuscript files have to be in RTF or Microsoft Word format (1.5 lines spacing, Times, font size 12 pt, left justified). Upon submission, figures (low resolution), and tables should be added on separate sheets following the text body (not imbedded within the text). Figure and table captions should be consecutively numbered using Arabic numerals (combined figure plates should contain subdivisions using capital letters) and include all necessary information detailed enough for understanding without consulting the main text. Abbreviations used in tables, drawings, and graphs should be explained in the respective caption. When designing your figures, consider that these appear in black and white in the printed version but in colour in the PDF version of your article.

Large tables must be considered and formatted as an electronic supplement (which will not appear in print). There is also the possibility to provide additional figures and video files as electronic supplement. These supplements must be mentioned in the manuscript.

Guidelines and format All manuscripts should have a title page with only title, author(s) plus address(es), and suggested running head.

Major articles should include title, author(s), authors' addresses, detailed abstract (at maximum about 10% of the length of text body), up to 10 key words in addition to the title, and in the following order introduction, materials and methods, results, discussion, acknowledgements, references (which can be followed by an appendix or appendices). The sections material and methods, results, and discussion can be combined or exchanged by other sections, if expedient. Start figure captions on a new page at the end of the manuscript. All scientific genus and species names must be in italics. Do not use italics in any other part of the text. Use small capitals for authors cited in text and in references. Do not use completely CAPITALIZED words or author names in any part of the text. Do not use any format of letters except of italics, bold, and Small Capitals. Avoid using footnotes. Do not use hyphenation. Throughout the manuscript never use tabs, nor double or more space typing. Only literature cited within the text shall be included in references. Multiple publications by an author or a team of authors within the same year should be indicated by a, b, c forth. Use “&” (not “and”) in the text when citing a reference by two authors and “et al.” when more than two authors are involved. Avoid comma between author(s) and year of publication except when author(s) of taxa is/are meant: e.g. Trichobatrachus robustus Boulenger, 1900 or Atelopus cruciger (Lichtenstein & Martens, 1856). Personal communications or unpublished data shall be cited as follows (examples): (W. Böhme pers. comm.) or (W. Böhme unpubl. data). Worldwide web sources have to be cited by author and year in references.

Shorter contributions are also accepted and should include title, author(s), address(es), abstract, key words, main text body, acknowledgements, references, and if appropriate electronic supplement. Generally, the text of shorter contributions should follow the structure of major articles; however, do not include headings with the exception of acknowledgements and references. These shorter contributions will appear as so-called Correspondence in the issue.

Molecular data submitted for publication to SALAMANDRA must be deposited at a recognised archive, such as the National Institute of Health’s GenBank (National Center for Biotechnology Information, NCBI, USA). Representative voucher specimens must be deposited in scientific collections. Accession numbers for molecular sequences and voucher specimens must be provided in the manuscript.

For correct style and format, especially of citations including World Wide Web citations, refer to one of our exemplified published publications provided below in the download links.

Example: Major article

Example: Correspondence

Nomenclatural acts Taxonomic works must follow the current International Code of Zoological Nomenclature. Descriptions of new species/subspecies must include at least designation of a preserved holotype, collection number, exact locality including political units, e.g. departments, altitude above sea level, and geographic coordinates (e.g. Isecheno, 00°12’39’’ N, 34°46’36’’ E, 1,550 m above sea level, southern Kakamega Forest, Kakamega District, Western Province, Kenya), detailed description of the holotype and a diagnosis (the diagnosis should compare the new species/subspecies with all closely related taxa). Include measurements, as usually used for the respective taxonomic group, of the holotype and photographs or detailed drawings of same specimen.

For proper registration of a new zoological taxon and a nomenclatural act in zoology, we require two specific statements to be included in your manuscript. In the Results section, the Life Science Identifier (LSID) should be listed under the new or changed species name, for example: Brookesia micra sp. n.

ZooBank LSID: urn:lsid:zoobank.org:act:D1A239D6-93E8-4C34-A428-F79A2C8B6405

You will need to contact ZooBank to obtain a LSID. Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final paper.

Please also insert the following text into the methods chapter, in a paragraph to be called

"**Nomenclatural acts**": The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub: XXXXXXX. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: www.salamandra-journal.com.

Ethics Contributions based on permanent removal of specimens from the wild must be in agreement with respective national and international regulations and have to indicate the localities, collections, and catalogue numbers of voucher specimens. The numbers of requisite permits and licenses must be mentioned in the acknowledgements.

Authorship implies responsibility. All authors must have played a significant role in designing and performing the study and/or in writing the manuscript. Those whose efforts were limited solely to providing materials and financial support or commenting on the manuscript, should be recognised in acknowledgements. Honorary authorship must be avoided. The corresponding author has to sign a copyright form on behalf of all authors, implying that all authors agree with the publication in the submitted form and no competing interest are known.

Review process Editors will conduct a pre-check with respect to suitability of publication in the journal. If the required format and other formalities are not followed, the editor may reject the manuscript even when its content is suitable and ask for re-submission after modification. When appropriate, the editor will send the manuscript to two peer reviewers, which are not necessarily those suggested by the authors. In case of a certain conflict, authors may ask the editor to exclude a potential reviewer. Acceptance or rejection of a manuscript will be based upon the recommendations of the reviewers and communicated by the editor.

After review (if not rejected), authors should prepare one document of the revised manuscript in the track changes mode, a second document with all applied changes included and a rebuttal letter referring to the reviewer's and editor's comments in detail. All files have to be sent back to the responsible editor.

After final acceptance of the manuscript, photographic pictures must be send to the editors as electronic files in high resolution TIFF or JPEG format with minimum width of 200 mm at 300 dpi; line drawings, maps and diagrams must be submitted as TIFF or JPEG with at least 800 dpi at 200 mm width. Figures and tables have to be numbered. Make sure that text and numbers within figures are large and clear enough, even if reduced to column width in printing.

Page proofs are sent to authors by the layouter. Authors are committed to answer questions asked by the layouter and modify text and figures accordingly, if required. Publication is attached to publication fees, depending on DGHT-membership.

After being published, the corresponding author will receive a notice to download a high resolution PDF of the article from the SALAMANDRA-homepage.

ANEXO 3

Instructions for authors - Amphibia-Reptilia Journal

Publication of the Societas Europaea Herpetologica

Scope Amphibia-Reptilia (AMRE) is a leading European multi-disciplinary journal devoted to most of the aspects of herpetology: ecology, behaviour, evolution, conservation, physiology, morphology, palaeontology, genetics, and systematics of amphibians and reptiles. Amphibia-Reptilia publishes high quality original papers, short-notes, reviews, book reviews and news of the Societas Europaea Herpetologica (SEH). The SEH website is located at: seh-herpetology.org. Thomson Scientific's Journal Citations Report for 2015 ranks Amphibia-Reptilia with an Impact Factor of 1.396.

Ethical and Legal Conditions Please note that submission of an article for publication in any of Brill's journals implies that you have read and agreed to Brill's Ethical and Legal Conditions. The Ethical and Legal Conditions can be found here: brill.com/downloads/conditions.pdf.

Online Submission AMRE uses online submission only. Authors should submit their manuscript online via the Editorial Manager (EM) online submission system at: amre.editorialmanager.com/. First-time users of EM need to register first. Go to the website and click on the "Register Now" link in the login menu. Enter the information requested.

When you register, select e-mail as your preferred method of contact. Upon successful registration, you will receive an e-mail message containing your Username and Password. If you should forget your Username and Password, click on the "Send Username/Password" link in the login section, and enter your first name, last name and email address exactly as you had entered it when you registered. Your access codes will then be e-mailed to you.

Prior to submission, authors are encouraged to read the 'Instructions for Authors'. When submitting via the website, you will be guided stepwise through the creation and uploading of the various files. A revised document is uploaded the same way as the initial submission. The system automatically generates an electronic (PDF) proof, which is then used for reviewing purposes. All correspondence, including the editor's request for revision and final decision, is sent by e-mail.

Choosing Editors in EM During the submission process, authors will be requested to select one of the two co-editors of the journal, according to the subject area of the manuscript:

General Biology, Genetics and Systematics of Amphibians: Dr. Sebastian Steinfartz - Department of Evolutionary Biology, Unit Molecular Ecology, TU Braunschweig s.steinfartz@tu-bs.de

General Biology, Genetics and Systematics of Reptiles: Dr. Sylvain Ursenbacher - Department of Environmental Sciences, Section of Conservation Biology, University of Basel s.ursenbacher@unibas.ch

Each co-editor can assign the manuscripts to himself, to another co-editor or to an associate editor. When handled by an associate editor, all communications finally go through the co-editor in charge of the manuscript. Editors must be contacted using Editorial Manager.

File Formats The submission must consist of a single text file (.doc, .docx, .odt) for the text, tables and figure legends. For figures, .eps, .jpeg, .tiff, .gif, .pdf or .doc files should be used (with one figure per page).

Figures should not be embedded in an MS Word file but in their initial software. A figure containing several parts must be saved as a single file. To guarantee good resolution in printing, colour figures should be saved as an original .tiff or .eps file with an original resolution of 600 or 1200 dpi. Files for colour figures should be submitted in CMYK and not in RGB format.

If tables create problems with their placement within the main MS Word file, they can be uploaded separately. All hyperlinks and field codes (e.g., from bibliographic databases) must be removed. Manuscripts in which the track change procedure of MS Word was used must be carefully checked for final acceptance of all corrections and removal of marginal comments.

One pdf of all the separate files will be automatically generated by Editorial Manager.

Review Process All manuscripts that are not editorially rejected or sent back for correction according to the instructions for authors are sent out for peer review. Manuscripts are usually reviewed by at least two external reviewers, one of the two co-editors and possibly an associate editor. Both external reviewers and members of the advisory editorial board can be selected to review a paper. Reviewers are given five days to take in charge a manuscript and then a maximum of three weeks to return their comments via the web platform. The first decision is usually made within one to two months of receipt. Authors must resubmit their manuscript within six weeks of receipt of the decision letter (4 weeks for subsequent submissions), except in cases of personal arrangements made with the co-editors. In sending their revision, authors must provide a separate letter (reply to referees letter) in which they paste comments of the reviewers and their responses directly under each point raised. Revised manuscripts can be sent to reviewers again.

The average time from submission to publication is currently only 6 months. It is expected to be even shorter in the near future, when individual articles will be become available online in advance of the journal issue.

Supplementary Media / Data Files To support and enhance your manuscript, AMRE accepts electronic supplementary material, including supporting applications, high-resolution images, background datasets, sound or video clips, large appendices, data tables and other items that cannot be included in the article PDF itself. Authors should submit the material in electronic format together with the other manuscript files and supply a concise and descriptive caption for each file. In order to ensure that your submitted material is directly usable, please provide the data in one of the broadly accepted file formats for video, audio, etc. and limit the file size (e.g., for video: max. 3 GB). Supplementary files supplied will be published online at FigShare (www.figshare.com), to which reference is made in the published article on Brill Online Books and Journals, and vice versa.

Contact Address For any questions or problems relating to your manuscript please contact: amre@brill.com. For eventual questions about Editorial Manager, authors can also contact the Brill EM Support Department at: em@brill.com.

Submission Requirements

Types of Contributions

There are three categories of papers:

Reviews must be written by specialists in the field and focus on hot topics or subjects not reviewed recently in the literature.

Manuscripts that are solely descriptive; purely faunistic (e.g., species check-list); based only on captive breeding; consisting only of a juxtaposition of non-connected fields; based on a too small a sample size; or contain reports of work that appear to contravene accepted principles of conservation or ethical standards, may be rejected without external review. Moreover, the research must adhere to the legal requirements of the country in which the work was carried out. Papers describing new species are more likely to be considered if they offer broad discussion, present several new species, and are based on a sufficient number of specimens. Otherwise editorial rejection may apply.

Manuscripts that do not follow the editorial style will not be considered for publication and will be sent back to the authors.

The latest instructions for authors are available from the Editorial Manager platform (amre.edmgr.com), but also directly from seh-herpetology.org/amphrept/instructions and from brill.com/files/brill.nl/specific/authors_instructions/AMRE.pdf. Recent issues of Amphibia-Reptilia may also be consulted before submitting a manuscript. Some papers are available without charge on the BrillOnline Platform for the Journal at booksandjournals.brillonline.com/content/15685381.

Language Manuscripts should be in English, using British spelling and grammar. Spelling should be consistent throughout. If English is not an author's first language, authors may consult an English native speaker to improve and check the language of their manuscript.

Length Articles cannot be longer than 8000 words (with 6 tables or figures), short notes are limited to 3000 words (2 tables or figures) and reviews to 12 000 words. If authors feel that manuscripts intended as an article will suffer severely from the requested word count threshold, they should contact one of the respective co-editors prior to submission (pre-submission inquiry) in order to find out whether an exception is justified or not in a specific case.

Manuscript Structure

General Manuscripts must be formatted using double-spacing, with wide margins (3 cm), and with continuous page and line numbering throughout the entire text.

It is mandatory that each manuscript is accompanied by a cover letter in which the authors state why their findings are new and important, and therefore should be published in Amphibia-Reptilia.

The first page of all manuscripts must contain the title in lower case letters, the first and last names of all authors (no initials; a coma separates each author name, including the two last ones), the affiliation and address of each author, including the e-mail address of the corresponding author (manuscripts without a valid e-mail address will not be considered), the type of manuscript (article, short-note or review) and the total number of words in the whole manuscript (reference list, captions, and tables included), and in the abstract.

Short notes must be prepared without dividing the text into sections, but must contain an abstract. Articles should be assembled in the following order (after the title page): abstract, keywords, introduction, materials and methods, results, discussion, acknowledgements, references, tables, figure legends (grouped together), figures (one per page). Figures should not be embedded in the manuscript file (see below).

Abstract The abstract should present a brief summary of the topic, including its aims, results and the relevance of the work. It should be presented in only one language (English) and be no longer than the recommended length (maximum 250 words for articles and reviews, 150 words for short notes).

Keywords Four to eight keywords must be presented after the abstract. They should be different from the words used in the title of the manuscript.

Headings The main headings are written in bold, the second level headings in italics.

New Paragraphs Paragraphs must be indented (except after headings) and not separated from each other by an empty line.

Italics The scientific names of species should be in italics.

Introduction The introduction should clearly state the objectives of the study and place it within the context of previous publications. Conceptual introductions are preferred over descriptive texts. The introduction should not merely describe a study species or group but give an overview of a more general topic in herpetology and possibly other organismic groups. In other words, a paper cannot be justified just because a species is threatened or because natural history data are lacking.

Materials and Methods These should be presented in a smaller font than the rest of the manuscript (e.g., Times New Roman 10 vs 12). Furthermore, they should be explained in enough detail to allow replication. The sample sizes and the number of independent replicates should be clearly stated. For experimental work details on both housing and observational conditions should be stated. Environmental conditions should be controlled as much as possible to avoid biased results. The exact dates or period of sampling and observation must be given. For studies based on a small number of study sites, the geographic coordinates must be indicated. Statistics should be explained in the methods, particularly when complex models are used.

Results Anecdotal results should not be presented unless they are of primary importance. Instead, they should be stated in the discussion section as personal observations. Results should focus on the main argument(s) of the manuscript. Comparisons should be tested statistically. Sample sizes should be clearly presented.

Discussion The results should be discussed in the context of the existing literature. The discussion should not focus only on the study species or group, but should be placed into the context of arguments about other model species to render it in a more conceptual and broad concept. The literature should be covered in sufficient detail for both the topic and the study group. Each paragraph should focus on a different idea, but very short paragraphs should be combined with other paragraphs. The discussion must not be overly long. Speculation should be avoided.

References

Text Citations These should be presented in chronological order as follows: Petranka (1998) or (Griffiths, 1996; Michimae and Wakahara, 2001; Schmidt, Feldman and Schaub, 2005). Where there are more than three authors, only the first should be named, followed by “et al.” (not in italics). Both the introduction and discussion must include an adequate number of citations for effective arguments to be established.

Reference List In the list, references should be listed in alphabetical, and then chronological order, under the first author’s name and should refer only to publications cited in the text. List references with three or more author names must be placed after those with two. Journal names must be abbreviated according to the official abbreviation. Many abbreviations are, for instance, available at: cassi.cas.org/search.jsp. No space must be inserted between the initials of the first names. No empty lines must be inserted between references. Volume numbers are written in bold. The two last authors or editors in a citation are separated only by a comma. Abstracts of conferences should not be listed in the reference list, but cited in the text as unpublished data or personal observation. The references of the species description (e.g. Linnaeus, 1758) are not necessarily included in Reference List. References must be typed in the following order and form, respectively:

- Arnold 2002
- Arnold 2003
- Arnold, Peterson 2002
- Arnold, Pfrender, Jones 2001
- Myers, E.M., Zamudio, K.R. (2004): Multiple paternity in an aggregate breeding amphibian: theeffect of reproductive skew on estimates of male reproductive success. Mol. Ecol. 13: 1951-1963.
- Kiesecker, J.M. (2003): Invasive species as a global problem. Toward understanding the worldwide decline of amphibians. In: Amphibian Conservation, p. 113-126. Semlitsch, R.D., Ed., Washington, Smithsonian.
- Zug, G.R., Vitt, L.J., Caldwell, J.P. (2001): Herpetology. An Introductory Biology of Amphibians and Reptiles, 2nd Edition. San Diego, Academic Press.

The use of bibliographic software such as Endnote is recommended to format the references correctly. An example of Endnote style file for Amphibia-Reptilia is available at sehherpetology.org/amphrept/instructions.htm. However, all Endnote fields (appearing in grey) must be removed from the text before submission. Independently of using such software, all references must be checked one by one accordingly to our guidelines. In particular, a great deal of attention needs to be paid to the abbreviations of journal names, as they do not depend directly on the downloadable style sheet.

Acknowledgements These should be kept brief, but funding agencies should be listed. If legal requirements are necessary for the study, the collecting permits must be cited with reference to the institution who issued them. Individuals are identified by their last name and the initials of their first name.

Statistics Means and standard errors (SE) / deviations (SD) or medians and quartiles or ranges should be given as: mean ± SE = 5.3 ± 0.3 mm. If equations or special symbols such as the mean are used, the module MS Equation in MS Word must be used (available in “Insert Object”). Statistical symbols, such as n, F, t, U, Z, r must be indicated in italics. Degrees of freedom are indicated as a subscript to the test statistic (F2, 265, t17). The name of the test should be given on its first appearance in front of the symbol (e.g., ANOVA, MannWhitney). The same test should be applied to the same kinds of analyses throughout the manuscript. P values for significant results should be quoted as below a threshold significance value ($P < 0.05$, $P < 0.01$, $P < 0.001$). Exact probabilities should be given for non-significant results (e.g. $P = 0.76$). Multiple post-hoc tests must be used with caution to avoid experimental error by chance alone. When transformations are used, they should be stated in the materials and methods. The multiple use of individuals should be controlled for or avoided. Multivariate analyses are usually requested when several explanatory variables are tested for one dependent variable or when one explanatory variable is expected to explain several dependent variables.

Abbreviations The International System of Units should be used. Do not employ unexplained abbreviations for institutions, etc.

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The Editors

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ANEXO 4

Instructions to Authors - The Herpetological Journal

The Britisch Herpetological Society

1) The Herpetological Journal publishes a range of features concerned with reptile and amphibian biology. These include:

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Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance. Authors should bear in mind that the Herpetological Journal is read by a wide range of herpetologists from different scientific disciplines. The work should therefore appeal to a general herpetological audience and have a solid grounding in natural history. General enquiries on suitability can be made to the Scientific Editor prior to submission.

2) All submissions are liable to assessment by the editorial board for ethical considerations, and publication may be refused on the recommendation of this committee. Contributors may therefore need to justify killing or the use of other animal procedures, if these have been involved in the execution of work. Likewise, work that has involved the collection of endangered species or disturbance to their habitat(s) will require full justification.

3) All submissions and illustrations should initially be sent by e-mail to the Scientific Editor (bhsherpetologicaljournal@gmail.com) as a single MS Word or PDF document, with a final size of 2 MB or less, containing the text and figures. At this stage, figures do not need to be at high resolution as long as the information contained in them can be clearly seen by the reviewers. If submission by e-mail is not possible, please contact the Scientific Editor for advice. All papers will be subject to peer review by at least two referees. Authors are asked to suggest the names of up to three suitable reviewers for their manuscript on submission, although the Editor reserves the right to use alternative referees. Papers will be judged on the basis of the reports supplied by referees, scientific rigour and the degree of general interest in the subject matter. The Editor's decision will be final.

4) Authors should consult a recent edition of the Journal for general guidance. Papers should be concise with the minimum number of tables and illustrations. Submitted manuscripts should be double spaced with wide margins all round. The usual rules of zoological nomenclature apply.

5) For all papers, the title page should contain only the following:

- Title of paper; name(s) and brief addresses of the author(s)
- A running title of eight words or less
- Up to six key words
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The text of the paper should begin on page 2 and be produced in the following order: Abstract, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections as appropriate. Footnotes are not permitted. Short Notes (generally less than six manuscript pages and accompanied by a single data set, i.e. one table or one figure) should be produced as continuous text, preceded by an abstract of no more than 100 words.

6) Papers should be written in UK English (including figure labels) and spelling should be that of the Oxford English Dictionary. Times and dates should conform to the following formats: for time of day use 0900, 1000 etc; for dates: 7 July 2008. Please avoid using bold text, all caps or small caps for emphasis or for headings. If emphasis is required, use italics.

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(Revised May 2017).

APÊNDICE 1

Manuscrito publicado no volume cinco, número um, do periódico Herpetologia Brasileira, seção de Notas de História Natural, páginas 16 e 17 (2016).

Título: *Scinax granulatus* (Amphibia, Anura, Hylidae): Multi-individual aggregation in a grove of exotic trees

Autoria: Ruth Anastasia Regnet e Daniel Loebmann

Número de palavras: 1.180

Qualis Capes 2017: C

Fator de impacto: Não possui

Scinax granulatus (Amphibia, Anura, Hylidae): Multi-individual aggregation in a grove of exotic trees

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Aggregation is a behavior observed in several vertebrate and invertebrate animal groups. Social insects (e.g., Hölldobler and Wilson, 1990), birds (e.g., Heppner, 1974; Miller, 1992) and migrating mammals (e.g., Frixell, 1995) are among the most well-known examples of animal aggregation. For amphibians, examples of aggregations are mainly reported for tadpoles (Brodie and Formanowiez, 1987; Glos et al., 2007; Smith and Awan, 2009), but it is also known for newly metamorphosed (Heinen, 1993) and adults (Hopkins and Lahanas, 2011). In adults, however, aggregation behavior can take place during reproduction, especially in species with explosive reproduction (Lynch and Wilczynski, 2006; Swanson et al. 2007; Knopp et al. 2008).

The *Scinax ruber* species group currently comprises 56 species, representing one of the most speciose clades within this genus (Faivovich et al. 2005). This is, among the Neotropical frogs, one of the group with the greatest taxonomic problems (Faivovich et al. 2005). From the state of Rio Grande do Sul, southern Brazil, four species of the *Scinax ruber* group have been reported: *Scinax fuscovarius* (Lutz, 1925), *Scinax granulatus* (Peters, 1871), *Scinax nasicus* (Cope, 1862), and *Scinax perereca* Pombal, Haddad and Kasahara, 1995 (Kwet, 2001).

Scinax granulatus occurs in the Atlantic Rainforest from Southern Brazil as well as all the way to the Pampas in Southern Brazil, Uruguay and northern Argentina (Haddad et al. 2013; Frost, 2015). It is a generalist species regarding habitat use, inhabiting lentic water bodies and their surroundings, being also found in anthropized areas (Maneyro and Carreira, 2012). During non-active periods, the frogs hide in and on bark of native and exotic trees, among grasses or taking advantage of artificial refuges as debris, heaps of stones or even inside homes (Maneyro and Carreira, 2012; Kwet et al. 2010). However, there is no information regarding multi-individual aggregation during non-active periods for species of the *Scinax ruber* group. Therefore, we report a record of aggregation of *S. granulatus* in a grove of exotic trees and discuss possible reasons for this behavior.

On October 16th, 2014 we performed a field trip at Universidade Federal do Rio Grande - FURG, municipality on Rio Grande, Rio Grande do Sul, Brazil (32°04'42.79" S, 52°10'09.45" W, 10 a.s.l.). Our prior goal was to collect data of amphibian species inhabiting the University campus. The collecting permit was issued by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, Proc. number 40540). This is an anthropized area where native vegetation has been partially replaced by alien trees, especially *Acacia mearnsii* De Wild., *Pinus elliottii* Engelm. and *Eucalyptus* sp.. In an individual of *P. elliottii* with ca. 18 m height near a temporary water body, we observed an aggregation of 23 individuals of *S. granulatus* (four females (SVL min - max = 2,43 – 2,93, SD = 0,21), eighteen males (SVL min - max = 2,07 – 3,44, SD = 0,33) and one unsexed individual that fled before measurements could be taken) using a plastic bag, hanging close to the tree bole, as a collective shelter. The bag was suspended ca. 1.50 m from the floor and contained plastic waste inside. Considering the high amount of feces present in the bag, it is reasonable to assume that those individuals were using it as shelter for a long period.

Examples of aggregations aiming to share refuges are poorly documented and the causes for this behavior can be interpreted as a strategy to avoid dehydration (Heinen, 1993) or to reduce predation risks (Graves et al. 1993; Hopkins and Lahanas, 2011). We speculate that the aggregation of *S. granulatus* herein reported could be associated with the former cause. Moreover, anthropic influence on the habitat as replacing native vegetation by introduced exotic trees, drastically reduces available microhabitats. It may also deeply influence the hydrologic balance. Finally, considering that *P. elliottii* produces substances that may be toxic for many species, it is possible that *S. granulatus* may not be able to survive hidden on bark of *P. elliottii,* forcing individuals to aggregate in artificial shelters.

Acknowledgments

We are grateful to Guilherme Sampaio Sansão for reviewing grammar and style of the short note. RAR thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for a MSc. fellowship.

Reference

Brodie Jr, E.D. and D.R. Formanowicz Jr. 1987. Antipredator mechanisms of larval anurans: protection of palatable individuals. Herpetologica, 43: 369-373.

Faivovich, J., C.F.B. Haddad, P.C.A. Garcia, D.R. Frost, J.A. Campbell. and W.C. Wheeler. 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. Bulletin of the American Museum of Natural History, 294: 1-240.

Frost, D.R. 2015. Amphibians Species of The Wolrd 6.0, an online reference. American Museum of Natural History. 20/09/2015, http://research.amnh.org/herpetology/amphibia/index.php.

Fryxell, J.M. 1995. Aggregation and migration by grazing ungulates in relation to resources and predators. pp. 257-273. In: Sinclair, A.R.E. & P. Arcese. (Eds), Serengeti II; Dynamics, Management, and Conservation of an Ecosytem; University of Chicago Press, Chicago.

Glos, J., G. Erdmann, K.H. Dausmann and K.E. Linsenmair. 2007. A comparative study of predator - induced social aggregation of tadpoles in two anuran species from western Madagascar. The Herpetological Journal, 17: 261-268.

Graves, B.M., C.H. Summers and K.L. Olmstead, 1993. Sensory mediation of aggregation among postmetamorphic *Bufo cognatus*. Journal of Herpetology, 27: 315-319.

Haddad, C.F.B., L.F. Toledo, C.P.A. Prado, D. Loebmann, J.L. Gasparini and I. Sazima. 2013. Guia dos anfíbios da Mata Atlântica – diversidade e biologia. Anolis Books. São Paulo. 542pp.

Heinen, J.T. 1993. Aggregations of newly metamorphosed *Bufo americanus* - tests of two hypotheses. Canadian Journal of Zoology, 71(2): 334-338.

Heppner, F.H. 1974. Avian flight formations. Bird-Banding, 45(2): 160-169.

Hölldobler, B. and E.O. Wilson. 1990. The Ants. Belknap Press. Cambridge. 746pp.

Hopkins, G.R. and P.N. Lahanas. 2011. Aggregation behaviour in a neotropical dendrobatid frog (*Allobates talamancae*) in western Panama. Behaviour. Leiden, 148(3): 359-372.

Maneyro, R. and S. Carreira. 2012. Guia de anfíbios del Uruguay. Colección Ciencia Amiga. Ediciones de la Fuga. Uruguay. 207pp.

Miller, R.C. 1922. The significance of the gregarious habit. Ecology, 3(2): 122-126.

Kwet, A., R. Lingnau and M. Di-Bernardo. 2010. Pró-Mata: Anfíbios da Serra Gaúcha, sul do Brasil - Amphibien der Serra Gaucha, Sudbrazilien - Amphibians of the Serra Gaúcha, South of Brazil. Brasilien Zentrum. University of Tübingen, Germany. 148pp.

Knopp, T., M. Heimoverta, H. Kokko and J. Merila. 2008. Do male moor frogs (*Rana arvalis*) lek with kin? Molecular Ecology, 17: 2522-2530.

Lynch, K. and W. Wilczynski. 2006. Social regulation of plasma estradiol concentrations in a female anuran. Hormones and Behavior, 50(1): 101-

106.https://archive.org/search.php?query=publisher%3A%22New+York%2C+Hafner+Pub.+Co.%22

Smith, G.R. and A.R. Awan. 2009. The Roles of Predator Identity and Group Size in the Antipredator Responses of American Toad (*Bufo americanus*) and Bullfrog (*Rana catesbeiana*) Tadpoles. Behaviour, 146(2): 225-243.

Swanson, E.M., S. M. Tekmen and M.A. Bee. 2007. Do female frogs exploit inadvertent social information to locate breeding aggregations? Canadian Journal of Zoology, 85(9): 921-932.

APÊNDICE 2

Manuscrito publicado no periódico The Herpetological Bulletin - The British Herpetological Society.

Título: *Leptodactylus latrans* tadpoles predating the eggs and tadpoles of sympatric anurans

Autoria: Ruth Anastasia Regnet e Daniel Loebmann

Número de palavras: 2.187

Figuras: 2

Qualis Capes 2017: B3

Fator de impacto: Não possui

Leptodactylus latrans tadpoles predating the eggs and tadpoles of sympatric anurans

RUTH ANASTASIA REGNET[1,2] and DANIEL LOEBMANN[1]

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ABSTRACT- The Criolla frog, *Leptodactylus latrans*, is one of the most common and widely distributed anuran species in the Neotropics. The tadpole of this species is among the largest within the Pampa Biome. In this study we examined feeding behaviour of *L. latrans* in the laboratory. Our results indicate that *L. latrans* tadpoles are potential predators of both larvae and eggs of other anurans.

INTRODUCTION

Anuran larvae are in general known to be primarily herbivores or detritivores; however, some species are carnivorous (Alford, 1999) although species are cannibalistic (Duellman & Trueb, 1994), and usually associated with the high density of tadpoles in water bodies (*e.g. Scaphiopus* spp., *Bombina variegata*, *Epidalea calamita* and *Hyla arborea*) (see Heusser, 1971; Pomeroy, 1981). For many species of phytotelmata breeders, low food availability induces females to lay unfertilized eggs to assist tadpole development (*e.g. Anomaloglossus beebei*, *Aparasphenodon arapapa*, *Oophaga pumilio*) (see Pramuk & Hiler, 1999; Bourne et al., 2001; Lourenço-de-Moraes et al., 2013). Some species breed in temporary ponds producing carnivorous tadpoles in order to obtain high-protein resources, accelerating metamorphosis and decreasing the risk of death by desiccation (Heusser, 1970; Crump, 1992;

Petranka & Thomas, 1995). Contrary to what is believed for phytotelmata breeders, carnivory in temporary ponds is considered opportunistic behaviour, occurring when individuals prey on eggs or dead tadpoles of heterospecific or conspecific animals (Petranka & Thomas, 1995; Haddad et al., 2005; De Sousa et al., 2014). However, carnivorous tadpoles are rare; one of the most conspicuous examples is within the *Ceratophrys* genus (Fernández & Fernández, 1921; La Marca, 1986). For leptodactilid tadpoles, carnivorous forms are unusual but two are known; *Leptodactylus labyrinthicus* (Spix, 1824) and *Leptodactylus pentadactylus* (Laurenti, 1768) (Laurenti, 1768; Silva & Giaretta, 2008; Piraini et al., 2010).

Leptodactylus latrans (Steffen, 1815) is one of the most widely distributed Leptodactylidae species in South America (Maneyro & Carreira, 2012; Pimenta et al., 2014). Its reproductive mode is classified as type 11 by Haddad & Prado (2005). Females are known to exhibit parental care over tadpole shoals during almost their entire development (Kwet et al., 2010; Maneyro & Carreira, 2012, Pimenta et al., 2014), which lasts from 50 to 60 days (Maneyro & Carreira, 2012; Heredia, 2008). Previous studies have shown tadpoles are omnivorous feeding on algae and animal remains (Lajmanovich, 1994) but to date there is no evidence that tadpoles of this species actively feed on other animals. However, our observations reveal that *L. latrans* tadpoles have the capacity to actively prey upon smaller tadpoles of *Physalaemus*. In the present work, we tested the hypothesis that tadpoles of *L. latrans* act as predators of eggs and smaller tadpoles of sympatric anuran species using an *ex situ* approach.

MATERIALS AND METHODS

To perform the experiment, we used nine *L. latrans* tadpoles, which were split into two size groups. The first group, hereafter named 'smaller' tadpoles, ranged from 40.1 to 46.2 mm total length (stage (S) 36, Gosner, 1960) and the second group, hereafter named as 'larger' tadpoles, ranged from 69.8 to 71 mm total length (S 40). These were offered fertilized eggs of *Physalaemus biligonigerus* (Cope, 1861) and tadpoles of *P. biligonigerus* in two development stages (mean = 5.79 mm total length, S 22; and mean = 5.90 mm total length, S 25); and tadpoles of *Rhinella fernandezae* (Gallardo, 1957) (mean = 5.67 mm total length, S 25). These were chosen because they are syntopic with *L. latrans* tadpoles in the coastal plain of Rio Grande do Sul, southern Brazil. All individuals were collected in temporary ponds at the university campus of Universidade Federal do Rio Grande (FURG) (-33.075694°,-52.168390°, 7 m above sea level, Datum WGS 84). The collection permit was authorized by Instituto Chico Mendes de Conservação da Biodiversidade (Licence Number 43658-1).

Eggs and tadpoles were collected in the wild and no tadpole was submitted to starvation conditions. Photoperiod in the laboratory mirrored natural conditions, that is 12h/12h. Each tadpole of *L. latrans* was placed in a transparent container filled with 0.55 L of rainfall water (21-23°C). Each *L. latrans* tadpole was simultaneously offered 15 eggs of *P. biligonigerus*; 15 tadpoles of *P. biligonigerus* (S 22), 8 tadpoles of *P. biligonigerus* (S 25) and 15 tadpoles of *R. fernandezae* (S 25). Feeding behaviour of *L. latrans* tadpoles was observed during the first 30 min. but the total number of tadpoles and eggs consumed was counted only after 12 h. The number of tadpoles and eggs were counted, including those that were rejected, that is, those *P. biligonigerus* and *R. fernandezae* tadpoles that were partially consumed (see Fig. 1).

Analysis of size effect on feeding behaviour (smaller vs larger than 50 mm) on the number of consumed items, was by non-parametric test (Mann-Whitney U-test). To detect significant differences between the numbers of consumed items we used a nonparametric ANOVA for dependent samples. Values were considered statistically significant at $\alpha = 0.05$. Results were expressed in *mean* percentage (± standard deviation). Statistical analyses were performed using Statistica 7.0 software.

RESULTS

Smaller tadpoles consumed fewer prey compared to the larger individuals (*U* = 28.5, p < 0.001; see Table 1). Therefore, to compare feeding preferences we used only the larger group (>50 mm) and detected significant differences in consumption between different items offered using ANOVA Chi Square (df = 3, n = 5) = 12.62; p = 0.006. That is, larger tadpoles of *L. latrans* consumed all items offered but with higher mean values for *P. biligonigerus* (S 22) (96 ± 5.96%) and *R. fernandezae* (S 25) (90.7 ± 14.6%) and lower mean values for *P. biligonigerus* (S 25) (45 ± 16.8 %) and eggs of *P. biligonigerus* (28 ± 19.1 %) (Fig. 2). Out of 135 *R. fernandezae* tadpoles 19 feeding attempts were made but rejected, while only one *P. biligonigerus* tadpole was rejected, out of 207 (Fig. 1 and Table 1).

DISCUSSION

These results support our initial hypothesis that *L. latrans* tadpoles will attack and consume larvae and also the eggs of other anurans. The lower predation capacity of *L. latrans* tadpoles with less than 50 mm total length may be attributed to limitations of smaller size. In many kinds of carnivorous and cannibalistic tadpoles, such behaviour is facultative and usually occurs under crowded conditions or when the food supply is limited (Crump, 1992; Duellman & Trueb, 1994). Although we found indications of predatory behaviour in *L. latrans* tadpoles there are no morphological adaptations for carnivory in their mouth apparatus, as previously observed in other carnivorous species of the *L. pentadactylus* group (Heyer, 1975). The findings in relation to *R. fernandezae* tadpoles, suggest they may be unpalatable. *Rhinella* tadpoles, and related species are known to produce alcianophilic mucous that produces an unpleasant taste, a possible anti-predator adaptation (Gunzburger & Travis, 2005). The rejection of bufonid tadpoles, for instance, has been reported in cases where *L. pentadactylus* tadpoles avoided *Rhinella marina* tadpoles after seizing and killing them (Heyer et al., 1975).

It is important to emphasize that this is the first evidence of carnivory for a species from the *L. latrans* group. Similar behaviour was previously recorded for *L. labyrinthicus* and *L. pentadactylus*, both belong to the *L. pentadactylus* group, which are known for preying upon both con- and heterospecific tadpoles (Heyer et al., 1975; Cardoso & Sazima, 1977; Wells, 1979; Silva & Giaretta, 2008; De Sousa et al., 2014). The ability to actively forage in order to obtain animal protein may be an essential component of a tadpole's development (Heyer et al., 1975) enhancing growth (Heusser, 1970; Crump, 1992; Petranka & Thomas, 1995). Also, carnivory could increase tadpole survivorship, particularly in scenarios such as small temporary ponds with limited food resources (Blair, 1976; Pomeroy, 1981; Crump, 1983). Our results, along with field observations, indicate that these tadpoles have the capacity to prey upon smaller tadpoles and anuran eggs, a behaviour that may influence survivorship rates of tadpoles of other anurans and small organisms that are syntopic.

ACKNOWLEDGMENTS

RAR are grateful to CAPES for providing a MSc. Scholarship. The authors are grateful to the comments of three reviewers, which improved the final version of the manuscript. We also thank Rebeca M. S. Alves, for improvements to the English grammar.

REFERENCES

Alford, R.A. (1999). Ecology: resource use, competition, and predation. In: *Tadpoles: The Biology of Anuran Larvae*, Pp 240-278. McDiarmid, R.W. & Altig, R. (Eds.). University of Chicago Press, USA.

Blair, F.W. (1976). Adaptation of anurans to equivalent desert scrub of North and South America. In: *Evolution of Desert Biota*, pp. 197-222. Goodall, D.W. (Ed.). Texas Press, Austin.

Bourne, G.R. Collins, A.C. Holder, A.M. & McCarthy, C.L. (2001). Vocal communication and reproductive behavior of the frog *Colostethus beebei* in Guyana. *Journal of Herpetology* 35: 272-281.

Cardoso, A.J. & Sazima, I. (1977). Batracofagia na fase adulta e larvária de rã-pimenta, *Leptodactylus labyrinthicus* (Anura: Leptodactylidae). *Ciência e Cultura* 29: 1130-1132.

Crump, M.L. (1983). Opportunistic cannibalism by amphibian larvae in temporary aquatic environments. *The American Naturalist* 121: 281-289.

Crump, M.L. (1992). Cannibalism in amphibians. In: *Cannibalism: Ecology and Evolution Among Diverse Taxa*, pp. 256-276. Elgar M.A. & Cresoi, B.J. (Eds.). Oxford University Press, USA.

De Sousa, V.T.T. Nomura, F. Venesky, M.D. Rossa-Feres, D.C. Pezzuti, T.L. Andrade, G.V. & Wassersug, R.J. (2014). Flexible feeding kinematics of a tropical carnivorous anuran tadpole. *Journal of Zoology* 293: 204-210.

Duellman, W.E. & Trueb, L. (1994). *Biology of Amphibians*. Baltimore: Johns Hopkins University Press 670pp.

Fernández, K. & Fernández, M. (1921). Sobre la Biología y Reproducción de Algunos Batracios Argentinos. 1° Edition. Cystignathidae. *Anales De La Sociedad Cientifica Argentina* 91: 97-193.

Gosner, K.L.A. (1960). A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. *Herpetologica* 16: 183-190.

Gunzburger, M. & Travis, J. (2005). Critical literature review of the evidence for unpalatability of amphibian eggs and larvae. *Journal of Herpetology* 39: 547-571.

Haddad, C.F.B. Faivovich, J. & Garcia, P.C.A. (2005). The specialized reproductive mode of the treefrog *Aplastodiscus perviridis* (Anura: Hylidae). *Amphibia-Reptilia* 26: 87-92.

Haddad, C.F.B. & Prado, C.P.A. (2005). Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brasil. *BioScience* 55: 207-217.

Heredia, J. (2008). *Anfibios de centro de Argentina/ Amphibians of central Argentina.* 1° Edition. Literature of Latin America, Argentina 100pp.

Heusser, H. (1970). Spawn eating by tadpoles as possible cause of specific biotype preferences and short breeding times in European anurans (Amphibia, Anura). *Oecologia* 4: 83-88.

Heusser, H. (1971). Laich Räubern und kannibalismus bei sympatrischen anurans Kaulquappen. *Experientia* 27: 474-475.

Heyer, R.W. McDiarmid, R.W. & Weigmann, D.L. (1975). Tadpoles, predation and pond habitats in the Tropics. *Biotropica* 7: 100-111.

Kwet, A. Lingnau, R. & Di-Bernardo, M. (2010). *Pró-Mata: Anfíbios da Serra Gaúcha, sul do Brasil - Amphibien der Serra Gaúcha, Südbrasilien - Amphibians of the Serra Gaúcha, South of Brazil.* 2° Edition. Brasilien Zentrum der Universität Tübingen, Tübingen.

Lajmanovich, R.C. (1994). Contribucíon al conocimiento de la alimentación de larvas de la rana criolla *Leptodactylus ocellatus* (Amphibia, Leptodactylidae) em el Paraná médio, Argentina. *Studies on Neotropical Fauna and Environment* 29: 55-61.

La Marca, E. (1986). Description of the tadpole of *Ceratophrys calcarata. Journal of Herpetology* 20: 459-461.

Laurenti, J.N. (1768). *Specimen Medicum, Exhibens Synopsin Reptilium Emendatam cum Experimentis Circa Venena et Antidota Reptilium Austriacorum.* Typis. Joan. Thomae nob. de Trattnern, AT. 214pp

Lourenço-de-Moraes, R. Lantyer-Silva, A.S.F. Toledo, L.F. & Solé, M. (2013). Tadpole, Oophagy, Advertisement Call, and Geographic Distribution of *Aparasphenodon arapapa* Pimenta, Napoli & Haddad 2009 (Anura, Hylidae). *Journal of Herpetology* 47: 575-579.

Maneyro, R. & Carreira, S. (2012). *Guía de anfíbios del Uruguay.* 1° Edition. Ediciones de la fuga. Coleccion Ciencia Amiga Press, Uy. 207pp.

Petranka, J.W. & Thomas. D.A.G. (1995). Explosive breeding reduces egg and tadpole cannibalism in the wood frog, *Rana sylvatica. Animal Behaviour* 50: 731-739.

Pimenta, B. Costa, D. Murta, R.F. & Pezzuti, T.L. (2014). *Anfíbios. Alvorada de Minas, Conceição de Dentro, Dom Joaquim.* Bicho do Mato Press, MG. Pp 196.

Piraini, R.M. Da Silva, E.T. & Feio, R.N. (2010). Tadpole cannibalism in *Leptodactylus cunicularius* Sazima & Bokermann, 1978 (Anura, Leptodactylidae) at a temporary stream in South-eastern Brazil. *Herpetology Notes* 3: 359-360.

Pomeroy, L.V. (1981). Characteristics and determinants of polymorphism in the larvae of the spadefoot toad, *Scaphiophus spp.* Phd. Thesis., U. California, Riverside.

Pramuk, J.B. & Hiler, B.I. (1999). An investigation of obligate oophagy of *Dendrobates pumilio* tadpoles. *Herpetological Review* 30: 219-221.

Silva, W.R. & Giaretta, A.A. (2008). Further notes on the natural history of the South American pepper frog, *Leptodactylus labyrinthicus* (Spix, 1824) (Anura, Leptodactylidae). *Brazilian Journal of Biology* 68: 403-407.

Wells, K.D. (1979). Reproductive behavior and male mating success in a Neotropical toad, *Bufo typhonius*. *Biotropica* 11: 301-307.

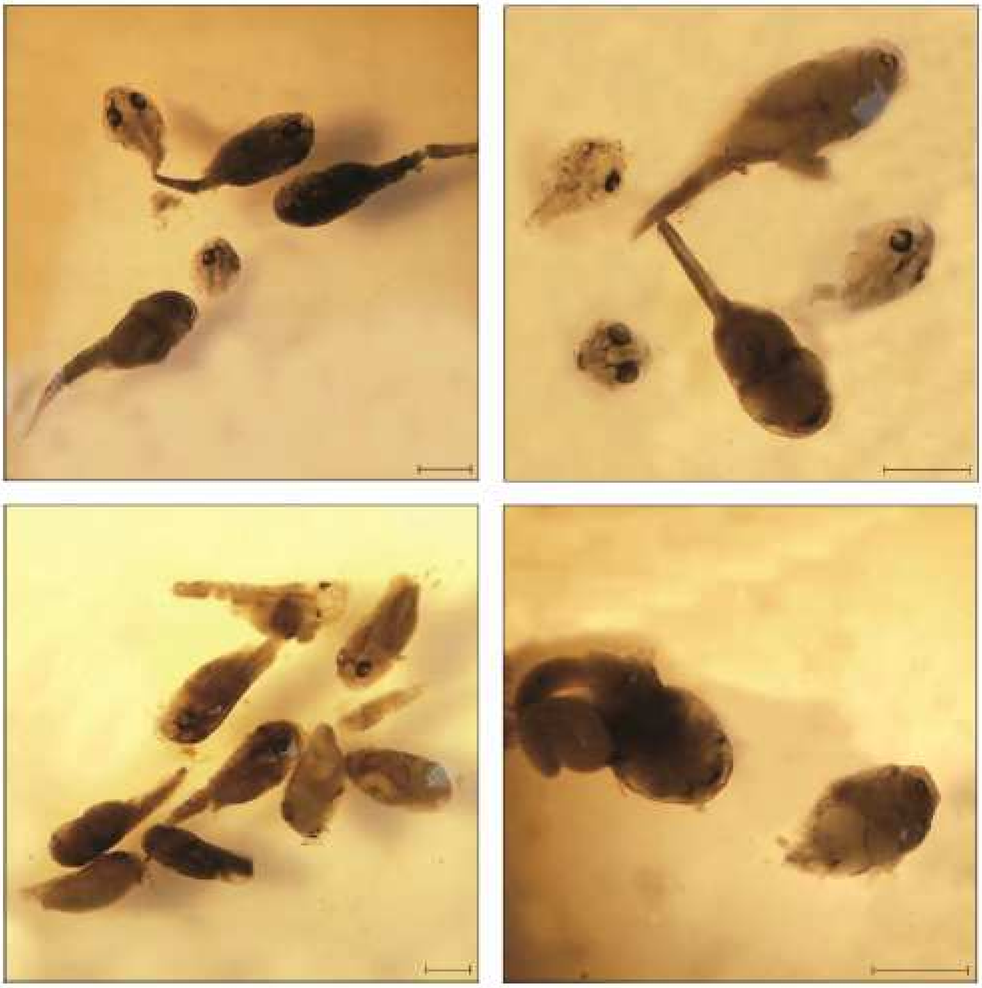

Figure 1. Examples of *Rhinella fernandezae* tadpoles found dead, partially destroyed or rejected by tadpoles of *Leptodactylus latrans* during the experiment. Scale bars represents 1 mm.

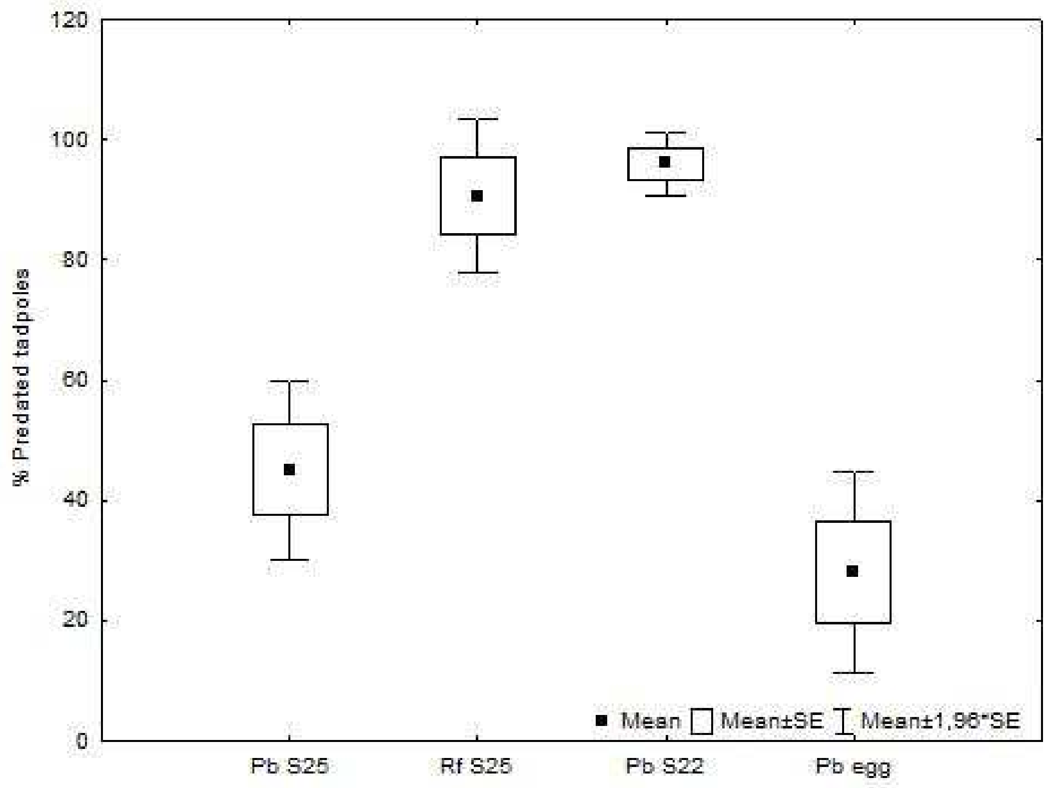

Figure 2. Mean, standard error and confidence intervals for the number of preys items consumed by larger *Leptodactylus latrans* tadpoles in the experiment. Pb = *Physalaemus biligonigerus*; Rf = *Rhinella fernandezae*; S = Gosner developmental stage.

APÊNDICE 3

Manuscrito aceito para publicação no periódico Journal of Herpetology.

Titulo: A new and microendemic species of *Ophiodes* Wagler, 1828 (Sauria: Diploglossinae) from the Lagoa dos Patos Estuary, southern Brazil

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Número de palavras: 4.318

Figuras: 5

Qualis Capes 2017: B1

Fator de impacto: 0,911

A new and microendemic species of *Ophiodes* Wagler, 1828 (Sauria: Diploglossinae) from the Lagoa dos Patos Estuary, southern Brazil

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LRH: Entiauspe-Neto et al.

RRH: New species of *Ophiodes* from southern Brazil

Abstract.—*Ophiodes* Wagler, 1828, is a poorly known legless lizard genus, widely distributed across South America east of the Andes, composed of five described species and other additional taxa that have not been formally described, but are widely referred in recent publications. After reviewing major herpetological collections in Rio Grande do Sul and conducting fieldwork for more than two decades in the Lagoa dos Patos Estuary, we came across a new species of *Ophiodes*, herein described. The new species is diagnosed from its congeners based on the combination of a dorsum with three wide dark brown longitudinal stripes and two pairs of conspicuous light-yellow stripes, a pair of one paravertebral and another dorsolateral; dark vertebral line absent; background coloration of sides light gray with four to five pale and narrow longitudinal stripes; ventral region uniformly light gray; hind limb extending to the posterior vent scale margin; small eyes, shorter than the half of snout-ocular distance; supralabials with well-defined, although small, supralabial blotches, restricted to their outer margins. We also provide comments on its distribution range and propose that a "Critically Endangered" CR B1b (i,ii,iii) extinction risk classification should be officially assessed and given.

Key words.—Taxonomy; Anguidae; South America; Pampa

Resumo.—*Ophiodes* Wagler, 1828, é um gênero de lagartos ápodos pouco conhecido, amplamente distribuído através da América do Sul à oeste dos Andes, composto de cinco espécies descritas e outros taxons que não foram formalmente descritos, embora sejam referenciados em publicações recentes. Após revisar coleções herpetológicas no Rio Grande do Sul e conduzir trabalho de campo por mais de duas décadas no Estuário da Lagoa dos Patos, encontramos uma nova espécie de *Ophiodes*, aqui descrita. A nova espécie é diagnosticada de seus congêneres com base na combinação de um dorso com três largas listras longitudinais marrom escuras e duas listras conspícuas amarelo-claras, um par composto de uma paravertebral e outra dorsolateral; linha vertebral escura ausente; coloração de fundo, nos lados, cinza claro com quatro à cinco listras longitudinais e estreitas; região ventral cinza claro uniforme; membro posterior estendendo-se a margem posterior da escama cloacal; olhos pequenos, menores que metade da distância entre focinho e olho; supralabiais com manchas bem definidas e pequenas, restritas à suas margens exteriores. Também apresentamos comentários sobre sua distribuição, e propomos que uma avaliação de risco de extinção "Criticamente em Perigo" CR B1b (i,ii,iii) seja oficialmente dada.

Palavras chave.—Taxonomia; Anguidae; América do Sul; Pampa

The Anguidae family encompasses 13 genera with approximately 131 living or extinct species, being distributed worldwide, recorded in Africa, Asia, Europe, North America, Central, and South America (Pianka and Vitt, 2003; Pyron et al., 2013; Uetz, 2016). The Anguidae subfamily Diploglossinae contains the genera *Celestus* Gray, 1839, *Diploglossus* Wiegmann, 1834, and *Ophiodes* Wagler, 1828, with approximately 51 species, distributed in the Caribbean Islands, Central America, and South America (Pianka and Vitt, 2003; Carreira et al., 2005; Vitt and Caldwell, 2014). Diploglossines usually present an elongated body, small but well developed limbs, and long, easily autotomic tails, with terrestrial, arboricolous, and fossorial habits (Vanzolini et al., 1980; Lema, 2002; Carreira et al., 2005; Vitt and Caldwell, 2014).

Ophiodes is distributed in most of South America east of the Andes, occurring in several biomes in Brazil, Bolivia, Paraguay, Argentina, and Uruguay (Peters and Donoso-Barros, 1970; Lema, 1994; Borges-Martins, 1998; Pizzatto, 2005); the genus is diagnosed by the lack of forelimbs, hind legs reduced to flaps, presence of osteoderms, a relatively long autotomous tail (up to twice its body length), and a relatively small head (Carreira et al., 2005; Barros and Teixeira, 2007).

Although stable as a genus, *Ophiodes* has had a intricate taxonomic history in regard to its species: Raddi (1820) presented the description of *Seps fragilis*, based on two specimens from Rio de Janeiro, southeastern Brazil. In the same year, Merrem (1820) presents the description of *Pygodactylus gronovii*. Five years later, Spix (1825) presented the description of *Pygopus striatus* and *P. cariococca*, both also from Rio de Janeiro. Boie (1826) synonimized *P. cariococca* and *P. striatus* with *Pygodactylus gronovii* Merrem, 1820.

Wagler (1828) revalidated *P. striatus*, describing the genus *Ophiodes* to place this species, while relegating *P. cariococca* to the synonymy of the former. Peters (1877) considered *P. striatus* and *P. cariococca* as synonyms of *Seps fragilis*. Bocourt (1881) presented the description of *Ophiodes vertebralis*, based on specimens from Uruguay and southern Brazil, diagnosed from *O. striatus* due to its dark vertebral line (absent in *O. striatus*) and black bars on the posterior portion of supralabial scutes (absent in *O. striatus*).

Boulenger (1893) described *Ophiodes intermedius* based on specimens from Paraguay, with a specific epithet referring to its intermediate characters between *O. striatus* and *O. vertebralis* (Cacciali and Scott, 2015). Boulenger (1913) also described *Ophiodes grilli* based on a single individual from Paraná, southern Brazil, distinguishing it from *O. striatus* based on a prefrontal contact with the second supraocular pair. Burt and Burt (1930) suggested that *O. vertebralis* and *O. grilli* should be considered subspecies of *O. striatus*, which was followed by Amaral (1937, 1949). Later, Vanzolini (1948) considered *O. grilli* as a synonym of *O. striatus*, while elevating *O. vertebralis* to a full species level.

Gallardo (1966) presented the most comprehensive revision to date of the genus, while providing a redescription of the genus *Ophiodes* and its species, describing *Ophiodes yacupoi* and presenting a key to the species in Argentina. Shortly after, Peters and Donoso-Barros (1970) recognized *O. intermedius*, *O. striatus*, *O. vertebralis*, and *O. yacupoi* as valid species. Hoogmoed and Grauber (1983) designated a lectotype and a paralectotype for *O. striatus* based on Spix's material, also highlighting that the name of Raddi (1820), *Seps fragilis*, should have priority over the former.

In a doctoral thesis with unpublished results, Borges-Martins (1998) presented an extensive revision of the genus, resurrecting *Ophiodes fragilis*, considering *O. yacupoi* as its synonym and presenting the identification of three new species; which are *Ophiodes* sp. “1” from central and southeastern Brazil, *Ophiodes* sp. “2” from southeastern and northeastern Brazil, and *Ophiodes* sp. “3” from southern Brazil, northeastern Argentina, Paraguay, and Uruguay (Cacciali and Scott, 2015). Cacciali and Scott (2015) later presented the description of *Ophiodes luciae*, based on a single preserved specimen, from Departamento Presidente Hayes, Paraguay.

In the state of Rio Grande do Sul, southern Brazil, Lema (1994) recorded *Ophiodes vertebralis* and *Ophiodes striatus*, suggesting that *O. striatus* was a very common species, and *O. vertebralis* was rare, being only seen in the southeastern portion of the state. The author also commented that *O. yacupoi* possibly occurs in the state, referring to two “phenotypes” that possibly constituted undescribed species. Lema (2002) would later only recognize *Ophiodes* cf. *striatus* and *Ophiodes fragilis* in the state. Quintela et al. (2006) presented records of *Ophiodes striatus* and *Ophiodes vertebralis* in the city of Rio Grande, in the southern region of the Rio Grande do Sul state, in the Lagoa dos Patos Estuary region. Later, Quintela & Loebmann (2009) and Quintela et al. (2011) refered to these specimens as *Ophiodes* sp.

While conducting a revision of *Ophiodes* specimens from Rio Grande do Sul, we came across the literature record of Quintela et al. (2006) labeled as “*Ophiodes vertebralis*”, as well as those of “*Ophiodes* sp.” aforementioned, and while examining its photographs and a specimen, observed a series of inconsistencies in its morphology regarding its attributed species. During a flooding caused by the El Niño climatic event at the Patos Lagoon Estuary, in the city of Pelotas, we collected a comprehensive series of specimens that could not fit the description of *Ophiodes vertebralis*, nor any other described taxa; these are herein described as a seemingly range-restricted new species, to which we provide comments on its geographical distribution and conservation status.

Materials and Methods

Body measurements were taken with a flexible ruler to the nearest millimeter. Bilateral variation is reported as left/right. Cephalic measurements were taken with digital calipers. Scale counts follow the methodology of Borges-Martins (1998). Measurements were taken to the nearest 0.01 mm. Acronyms follow Sabaj Pérez (2016), except for: CHFURG (Coleção Herpetológica da Fundação Universidade do Rio Grande - FURG) and MCN (Coleção Herpetológica da Fundação Zoobotânica do Rio Grande do Sul). We examined individuals and photographs in the MNHN (Uruguay), MNHN (France), ZVC-R, UFMG, MCN, MCP, and CHFURG collections; a list of individuals is presented on Appendix 1. Euthanasia was conducted with an intracardiac injection of a Xylocaine 1% solution to the heart; subsequently, specimens were fixated with 10% Formalin solution for 24 h, and then preserved in a solution of 75% Ethanol.

Results

Ophiodes enso sp. nov.

Ophiodes vertebralis –Lema, 1994:65; Quintela et al., 2006:61.

Ophiodes sp. –Quintela and Loebmann, 2009:40; Quintela et al., 2011:59.

(Figs. 1, 2A).

Holotype.—CHFURG 3589, an adult female, collected on 29 October 2015 by Daniel Loebmann (Figs. 1, 2), at Laranjal Beach (-31.7666º, -52.2166º, WGS84), altitude approximately 2 m, Patos Lagoon estuary, municipality of Pelotas, state of Rio Grande do Sul, Brazil.

Paratypes.—CHFURG 3564–3587 from type locality; CHFURG 3588 from Barra, municipality of Rio Grande (-32.0350º, -52.0988º); all specimens collected between 29 October and 3 November 2015 by Daniel Loebmann.

Examined Specimens.—MCN 15969 from Ilha dos Marinheiros, Rio Grande (-32.0000º, -52.1500º), collected by Fernando Marques Quintela on an unknown date of 2006.

Diagnosis.—*Ophiodes enso* sp. n. is distinguished from its congeners based on a combination of: (1) dorsum with three wide dark brown stripes and two pairs of conspicuous light-yellow lines, one paravertebral and another dorsolateral; (2) dark vertebral line absent; (3) lateral background coloration light gray with four to five pale and narrow longitudinal stripes; (4) ventral region uniformly light gray; (5) hind limb extending to the posterior vent scale margin; (6) small eyes, smaller than half of the snout-orbit distance; (7) supralabials with well-defined, although small, supralabial blotches, restricted to their outer margins; (8) 140–151 scales between occipital and vent; (9) supralabials 9/9; (10) supraciliaries 4/4; (11) 22–26 longitudinal scales around midbody. A table of comparative characters is provided (Table 1).

Ophiodes enso differs from *O. fragilis* by: 1) supralabials 1–5 with blackish posterior margins, being conspicuously barred (in *O. fragilis* supralabials with well-defined bars, and comparatively darker and longer stripes); 2) darker dorsal background coloration, with marked edges and lighter sides (dorsal and lateral coloration comparatively more uniform in *O. fragilis*); 3) two pairs of conspicuous dorsal light stripes (a single pair of less-defined dorsolateral light stripes is present in *O. fragilis*).

Ophiodes enso can be distinguished from *O. striatus* by: 1) supralabials with black blotches (well-defined supralabial bars in *O. striatus*); 2) flat supraocular region (raised supraocular region in *O. striatus*); 3) brownish lateral background (vividly greenish sides in *O. striatus*); 4) two preoculars (three preoculars in *O. striatus*); 5) 140–151 scales between occipital and vent (147–164 in *O. striatus*).

Ophiodes enso differs from *O. vertebralis* (Fig. 3) by: 1) absence of dark vertebral line (well-defined vertebral line in *O. vertebralis*); 2) presence of two conspicuous pairs of light yellow dorsal stripes (a single pair of conspicuous light yellow lateral stripes delimited by adjacent dark lines in *O. vertebralis*); 3) well-defined, although small, supralabial blotches, restricted to their outer margins (supralabials in *O. vertebralis* with inconspicuous and irregular black markings); 4) slender rostrum; (5) uniform dorsal head coloration (conspicuous black markings in *O. vertebralis*, while almost forming a stripe on its supraocular region).

Ophiodes enso is distinguished from *O. intermedius* by: 1) well-defined, although small, supralabial blotches, restricted to their outer margins (well-defined supralabial bars in *O. intermedius*); 2) absence of dark vertebral stripe (present in *O. intermedius*); 3) flat supraocular region (supraocular region raised in *O. intermedius*); 4) presence of two conspicuous pairs of light yellow dorsal stripes delimited by dark lines (a single pair of conspicuous light yellow dorsolateral stripes delimited by dark lines in *O. intermedius*).

Ophiodes enso differs from *O. luciae* by: 1) blotched supralabials (supralabials in *O. luciae* are uniformly colored), 2) flat supraocular region (conspicuously raised supraocular region in *O. luciae*); 3) comparatively smaller snout-orbit distance; 4) posterior appendage extending beyond the vent scale margin (posterior appendage short, not reaching the vent scale margin in *O. luciae*); 5) 140–151 scales between occipital and vent (171 in *O. luciae*); 6) supralabials 9/9 (supralabials 7/8 in *O. luciae*); 7) supraciliars 4/4 (supraciliars 5/5 in *O. luciae*); 8) 22–26 longitudinal scales around midbody (instead of 29 in *O. luciae*).

Description of Holotype.—A medium sized *Ophiodes*; adult female, snout-vent length (SVL) 206 mm, tail length 289 mm, tail length approximately 1.4 times SVL; body cylindrical; ear opening not visible; dorsal and dorsolateral scales (29 circumferential longitudinal rows at midbody of holotype) with longitudinal grooves; posterior appendage extending to the posterior vent scale margin.

Rostral rounded and well-developed, contacting the first supralabials, nasal, and internasal; nasal quadrate and short, smaller than the first supralabial; anterior internasal contacting rostral, nasal, and posterior internasal; posterior internasal slightly larger than anterior internasal, contacting anterior nasal, postnasal, anterior loreal and prefrontal; prefrontal large and heart-shaped, contacting posterior internasals, anterior loreal, posterior canthal, anterior supraocular, and frontal; frontal elongated, approximately twice as long as wide, contacting prefrontal, anterior supraocular, supraoculars 2–3, frontoparietal, and interparietal; frontoparietal small, similar in size to posterior supraocular; interparietal longer than wide, reaching approximately 0.6 frontal length, and contacting frontal, frontoparietal, parietal, and occipital; parietal longer than wide, similar in size to interparietal; occipital rhombus shaped, approximately half size of interparietal; supraoculars 5/5; supraciliars 4/4; postnasal contacting the posterior internasal and the first two supralabials; anterior loreal large, contacting supralabials 2 and 3; posterior loreal small, contacting supralabial 3; canthal wider than higher; preoculars 2/2; postoculars 3/3; temporals 4/4; supralabials 9/9, only the fifth contacting the eye; infralabials 9/9; symphyseal rounded; postsymphyseal wider than long, contacting only the first infralabials; two anterior gulars contacting the second infralabials; 3 posterior gulars; 4 sublabials; 141 ventral scales; 25 longitudinal scales around midbody; 6 scales along hind limb.

Color in Life.—Overall coloration pattern in life can be seen in Figures 1 and 4. Head dark brown except for paler supralabials, base of rostral and mandibular scales; posterior and upper margin of supralabials and preoculars (and temporal in some specimens) with slightly black bars. Three broad dark brown dorsal longitudinal stripes, one and two half scales wide and edged by a pair of thin black stripes. The dark brown dorsal stripes are interleaved with two pairs of light-yellow longitudinal stripes, limited by black lines. Sides light brownish gray with four to five pale brown lateral lines. Ventral region uniformly light gray. At the cloacal opening, the vertebral pair of light-yellow longitudinal stripes converges proximally and fades, while two new stripes emerge, without a black margin on its inner portion; the dark brown stripes turn into thin black stripes, over a light brown background. The pattern below the cloacal opening is not visible in specimens that had autotomized its tails, becoming indistinct dark brown or light gray.

It is also noteworthy that specimens from Rio Grande (CHFURG 3588, MCN 15696) presented, instead of dark brown longitudinal stripes, metallic beige stripes. Due to the lack of beige specimens from Pelotas, and to this being an exclusive pattern of the Rio Grande localities, we believe that this might be a phenotype restricted to the sandy habitats and salt-marshes from Rio Grande; further studies are needed in order to evaluate if the establishment of restricted color patterns may be a result of restricted gene flux or reduced populations.

Color in Preservative.—After a year in preservative, its coloration has remained very similar to in life; head and dorsal longitudinal stripes that were originally dark brown are black, while its adjacent light-yellow stripes have turned gray or white.

Variation.—We observed a range of 140–151 scales between occipital and vent, 22–26 longitudinal scales around midbody, and 6–8 scales along hind limb.

Etymology.—The species name refers to the Japanese-Buddhism Ensō (円相) symbol, a hand-drawn circle made in a single or double brushstroke, that closely resembles the silhouette of a *Ophiodes* specimen (Fig. 2). It is also noteworthy that the type series of *O. enso* sp. n. was discovered during the El Niño Southern Oscillation event of 2015 (popularly known as El Niño), which is marked by heavy rainfall, that culminated in the movement of the aforementioned type series into the Lagoa dos Patos shores. This is also the first species to be discovered as a result of the ENSO phenomenon.

Distribution and Natural History.—The new species is known only from three localities at the Lagoa dos Patos estuary, the municipalities of Pelotas (-31.7666º, -52.2166º), continental Rio Grande (-32.0350º, -52.0988º), and Ilha dos Marinheiros (32.0000º, -52.1500º), an islet of Rio Grande, all located at the Holocene sandbanks of the Coastal Plains of Rio Grande do Sul, in the Pampa biome (Fig. 5). A description of the vegetation and composition of the Lagoa dos Patos estuary is provided by Verrastro et al. (2003).

Conservation.— *O. enso* is known only from three localities, its estimated range is of less than 100 km² and the quality of habitat and area of occupancy are in continued decline, as there are also no protected areas throughout its range. Following IUCN guidelines (IUCN, 2016), we suggest listing *O. enso* as "Critically Endangered" CR B1b (i,ii,iii). We also highlight that the area in which most specimens were collected (Laranjal beach, near the "Pontal da Barra") is suffering from strong anthropogenic impacts, with the grounding of wetlands (already restricted to a small strip of less than 5 km of extent), in areas that are intended for the urban sites construction.

Discussion

The description of this new species elevates the *Ophiodes* diversity to six taxa. Unfortunately, not much has changed since the pioneering work of Gallardo (1966); the formal publication of a comprehensive revision is needed, in order to elucidate the issues of the three proposed undescribed species of Borges-Martins (1998), as well as the taxonomic status of species which have a confusing taxonomic status, such as *O. yacupoi*, and morphological limits of the contained species, that remain unevaluated.

The external morphology of the new species appears to be closely related with *O. vertebralis* (Figs. 3, 4), as it was previously confused with this species by former authors (e.g. Lema, 1994; Quintela et al., 2006), and although occurring in the same biome (Pampas), their geographical distributions do not overlap (Fig. 5). In the localities of Pelotas and Rio Grande, the authors have observed *Ophiodes striatus* (F. M. Q., pers. obs.), although it is not clear whether both species occur in syntopy, because *O. striatus* can be found in a wide array of habitats, with various degrees of environmental pressures; *O. enso* is probably restricted to the southernmost region of the Lagoa dos Patos estuarine range, being expected to occur strictly around estuarine habitats, such as saltmarshes and wetlands.

The Lagoa dos Patos estuary has been intensively sampled in the past 20 yr and only after this severe flooding episode it was possible to find several individuals of this new taxon, which drifted ashore. Despite sampling efforts in several adjacent municipalities (such as São José do Norte, Capão do Leão, São Lourenço do Sul and Morro Redondo), as well as approximately 50 and 100 km northward in the beaches from Laranjal, no other specimens were found, whereas to the south, a single individual was found, presumably drifted along the floating vegetation at the mouth of the Lagoa dos Patos, in the Rio Grande municipality, at a breakwater pier approximately 2 km into the ocean. Besides a single specimen reported by Quintela et al. (2006) to Ilha dos Marinheiros, a estuarine islet of the Rio Grande municipality, no other specimens were discovered while reviewing major collections.

As the influence of polymorphism and sexual dimorphism in species delimitation of this genus are still largely unknown, we urge other authors to refrain from publishing new descriptions based on small series or preserved series (such as the description of *O. luciae*, based on a single preserved specimen), as this may also contribute to the taxonomic instability in the genus, because some diagnostic features of the latter species may be a result of its preservation (for instance, its diagnostic uniform supralabial region may be heavily discolored from a long exposure to light and ethanol; Fig. 4). Further studies of population dynamics and geographical distribution are needed in order to verify the conservation status of *O. enso* sp. n., since it has a restricted range and is probably a threatened species, therefore assisting in the development of local environmental policies to conserve its range and associated habitat.

Acknowledgments.—The authors thank G. Surlo for providing color drawings of *Ophiodes* species and C. O. Gridi-Papp for reviewing the manuscript for grammar and style. FMQ thanks CAPES for granting a post-doctoral scholarship, OME-N thanks CNPQ for a PIBIC grant. We are also deeply indebted to N. Vidal and the Muséum National D' Histoire Naturelle curator staff for providing us photographs of the *O. vertebralis* syntypes under their care. A. D. Abegg significantly helped in making the map, collecting geographical coordinates and obscure references. We also thank two anonymous reviewers for their helpful suggestions. Collecting permits were issued by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) (Process 43658-1), and euthanasia methods were approved by the Comissão de Ética em Uso Animal (CEUA - FURG).

Literature Cited

Amaral, A. 1937. Estudos sobre lacertilios Neotropicos, 4. Lista Remissiva dos Lacertilios do Brasil. Memórias do Instituto Butantan 11:1–47.

Amaral, A. 1949. Lacertílios do Pará. Boletim do Museu Paraense Emílio Goeldi 10: 107–114.

Barros, E. H., and R. L. Teixeira. 2007. Diet and fecundity of the glass-lizard, *Ophiodes striatus* (Sauria, Anguidae), from the Atlantic Forest in southeastern Brazil. Boletim do Museo Biologico Mello Leitão 22: 11–23.

Bocourt, M. F. 1881. Études sur les reptiles et les batraciens. Pp. 441–488 in A. Duméril, M. F. Bocourt, and M. Mocquard (Eds.). Recherches Zoologiques pour servir à L'Histoire de la Faune de L'Amérique Centrale et du Mexique, publiées sous la direction de MM. H. Milne Edwards, membre de l'Institut et Léon Vaillant Professeur au Muséum. Imprimerie Impériale, France.

Boie, H. 1826. Bemerkungen uber die von Herm Von Spix abgebildeten brasilianischen Saurier. Isis van Oken 18: 117–120.

Borges-Martins, M. 1998. Revisão taxonômica e sistemática filogenética do gênero *Ophiodes* Wagler, 1828 (Sauria, Anguidae, Diploglossinae). Ph.D. Dissertation, Pontifícia Universidade Católica do Rio Grande do Sul, Brazil.

Boulenger, G. A. 1893. List of reptiles and batrachians collected by Dr. J. Bohls near Asuncion, Paraguay. Annals and Magazine of Natural History, London (Series 6) 13: 342–348.

Boulenger, G. A. 1913. Descriptions of a new lizard and a new snake from South America. Annali del Museo cívico di storia naturale di Genova 6: 49–50.

Burt, C. E., and M. D. Burt. 1930. The South American Lizards in the collection of the United States National Museum. Proceedings of the United States National Museum 78: 1–52.

Cacciali, P., and N. J. Scott. 2015. Key to the *Ophiodes* (Squamata: Sauria: Diplohlossidae) of Paraguay with the description of a new species. Zootaxa 3980: 42–50.

Carreira, S., M. Meneghel, and F. Achaval. 2005. Reptiles de Uruguay. Universidad de la República, Uruguay.

Gallardo, J. M. 1966. Las especies Argentinas del género *Ophiodes* Wagler. Revista del Museo Argentino de Ciencias Naturales Bernardino Rivadavia 9:123–144.

Hoogmoed, M. S. and U. Gruber. 1983. Spix and Wagler type specimens of reptiles and amphibians in the Natural History Museum in Munich (Germany) and Leiden (The Netherlands). Spixiana 9: 319–415.

Lema, T. 1994. Lista comentada dos répteis ocorrentes no Rio Grande do Sul, Brasil. Comunicações do Museu de Ciências da PUCRS, Série Zoologia, Porto Alegre 7: 41–150.

Lema, T. 2002. Os Répteis do Rio Grande do Sul: Atuais e Fósseis – Biogeografia – Ofidismo. EDIPUCRS, Brazil.

Peters, W. 1877. Herpetologische Notizen. I. Uber die von Spix in Brasilien gesammelten Eideschsen des Koniglichen Naturalien-Kabinets zu München. Monatsberichte der Königlichen Preussiche Akademie des Wissenchaften Zu Berlin 1877: 407–414.

Peters, J. A., and R. Donoso-Barros. 1970. Catalogue of the Neotropical Squamata, Part. II; Lizards and amphisbaenians. Bulletin of the United States National Museum 297: 1–293.

Pianka, E. R., and L. J. Vitt. 2003. Lizards: Windows to the Evolution of Diversity. University of California Press, United States of America.

Pizatto, L. 2005. Reproductive biology of the “glass snake” *Ophiodes fragilis* (Squamata: Anguidae) in South-East Brazil. Herpetological Journal 15: 9–13.

Pyron, R. A., F. T. Burbrink, and J. J. Wiens. 2013. A phylogeny and updated classification of Squamata, including 4161 species of lizards and snakes. BMC Evolutionary Biology 13: 93.

Sabaj Pérez, M. H. 2016. Standard Symbolic Codes for Institutional Resource Collections in Herpetology and Ichthyology: An Online Reference, version 6.5 [Internet]. American Society of Ichthyologists and Herpetologists, USA. Available from: http://www.asih.org/. Accessed 05 April 2017.

Quintela, F. M., D. Loebmann, and N. M. Gianuca. 2006. Répteis continentais do município de Rio Grande, Rio Grande do Sul, Brasil. Biociências 14: 180–188.

Quintela, F. M. and D. Loebmann. 2009. Os Répteis da Região Costeira do Extremo Sul do Brasil. União Sul-Americana de Estudos da Biodiversidade, Brazil.

Quintela, F. M., C. Ibarra, L. F. M. Neves and M. R. C. Figueiredo. 2011. Reptiles recorded in Marinheiros Island, Patos Lagoon estuary, southern Brazil. Herpetology Notes 4: 57–62.

Raddi, G. 1820. Di alcune specie nuovi di rettili e piante brasiliani. Atti ella Società Italiana di Scienze Moderna 18: 1–39.

Spix, J. B. 1825. Animalia Nova Sive Species Nova Lacertarum quas in Itinere per Brasiliam Annis MDCCCXVII-MDCCXX jussu et Auspiciis Maximiliani Josephi I. Bavariae Regis, Suscepto, Collegit et Descripsit Dr. J. B. de Spix. Typis Francis Seraphici Hübschmanni, Germany.

Uetz, P. 2015. The Reptile Database [Internet]. Available from http://www.reptile-database.org/. Accessed 24 November 2016.

Vanzolini, P. E. 1948. Notas sobre ofidios e lagartos da Cachoeira de Emas, no município de Pirassununga, Estado de São Paulo. Revista Brasileira de Biologia 8: 377–400.

Vanzolini, P. E. 1977. An annoted bibliography of the land and fresh-water reptiles of South America (1758-1975). Museu de Zoologia, Universidade de São Paulo, Vol. I, Brazil.

Vanzolini, P. E., A. M. Ramos-Costa, and L .J. Vitt. 1980. Répteis das Caatingas. Academia Brasileira de Ciências, Brazil.

Verrastro, L., L. Veronese, C. Bujes, and M. M. D. Filho. 2003. A new species of *Liolaemus* from southern Brazil (Iguania: Tropiduridae). Herpetologica 59: 105–118.

Vitt, L .J., and J. P. Caldwell. 2014. Herpetology: An Introductory Biology of Amphibians and Reptiles, 4th edition. Academic Press, United States of America.

Wagler, J. 1828. Auszuge aus seinem Systema Amphibiorum. Isis von Oken 21: 740–742.

Appendix 1

Material examined (Specimens marked with an asterisk refer to individuals of the Muséum National D' Histoire Naturelle (France) and were examined by photographs)

Ophiodes fragilis. BRAZIL: Unknown locality: (MNRJ 15564, MNRJ 15792, MNRJ 20455). Minas Gerais: Passa Vinte: (UFMG 1405, UFMG 1406). Simonésia: (UFMG 1080). Rio Preto: (UFMG 1785).

Ophiodes intermedius. Unknown locality: (MNHN 9394, MNHN 9393, MNHN 9395). ARGENTINA: Córdoba: Rio Cuarto: Campus Universidad Nacional Rio Cuarto: (ZVC-R 4352, 4353). URUGUAY: Paysandú: Unknown locality: (ZVC-R, 4428). Rincón de Constância: (ZVC-R 500). Salto: Salto: (ZVC-R 5482). Salto Grande: (ZVC-R 1999).

Ophiodes vertebralis. Unknown locality: (MNHN 5558*, MNHN 6159*, MHNH 1999.8065, Syntypes of *O. vertebralis**). ARGENTINA: Estancia El Toro-Las Flores (MNHN 1052). URUGUAY: Durazno: Santa Bernardina: (MNHN 1053). Canelones: Isla de Flores: (ZVC-R 1417). Maldonado: Barra de Maldonado: (ZVC-R 2784). Isla de Lobos: (ZVC-R 3352). Montevideo: Buceo: (ZVC-R 5935, MNHN 930). Calle Mataojo: ZVC-R 6242. Camino Carrasco: MNHN 720, 3567. Malvin: (MNHN 3806). Montevideo: (ZVC-R 4632). Cercanias del predio de Facultad de Ciencias: (ZVC-R 6173). Punta Carretas: (MNHN 931). Paysandú: Alrededores de la Ciudad de Paysandú: (MNHN 204). Playa Malvin: (ZVC R-17). Puerto Del Buceo: (ZVC-R-336). San José: Campo Experimental nº 2, Ruta 1, Km 42: (ZVC-R 4653).

Ophiodes striatus. BRAZIL: Minas Gerais: Buenópolis: (UFMG 2014). Minduri: (UFMG 2405).

Table 1. Diagnostic characters in *Ophiodes*, modified from Cacciali and Scott (2015). Unknown character states are indicated with an interrogation (?) mark.

	Scales between occiput and vent	**Scales around midbody**	**Scales on hind limb**	**Supralabial region**
Ophiodes enso sp. n.	140–151	22–26	6–8	Well-defined, although small, supralabial blotches, restricted to their outer margins
Ophiodes luciae	171	29	4	Uniformly colored
Ophiodes intermedius	139–158	25–27	5–7	Well-defined supralabial bars
Ophiodes vertebralis	136–150	25–28	4–7	Inconspicuous and irregular black markings
Ophiodes striatus	147–164	24–27	5–8	Well-defined supralabial bars
Ophiodes fragilis	130–147	23–29	6–10	Well-defined bars, and comparatively darker and longer stripes than its congeners
Ophiodes sp. "1"	142–156	24–27	3–6	?
Ophiodes sp. "2"	135–153	23–26	5–6	?
Ophiodes sp. "3"	144–161	24–27	5–9	?

Figures

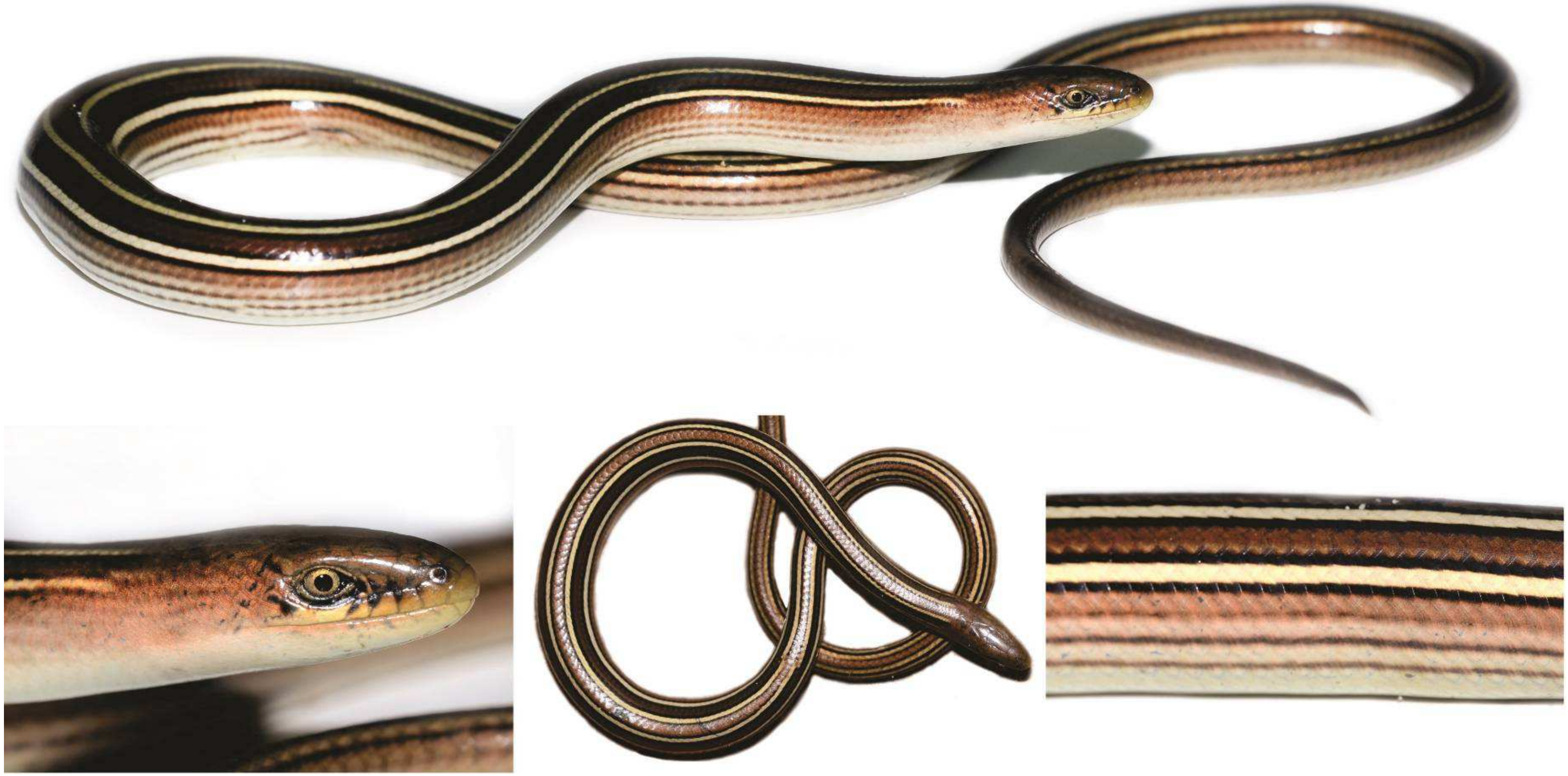

Fig. 1. Holotype of *Ophiodes enso* sp. n. (CHFURG 3589) exhibiting coloration in life

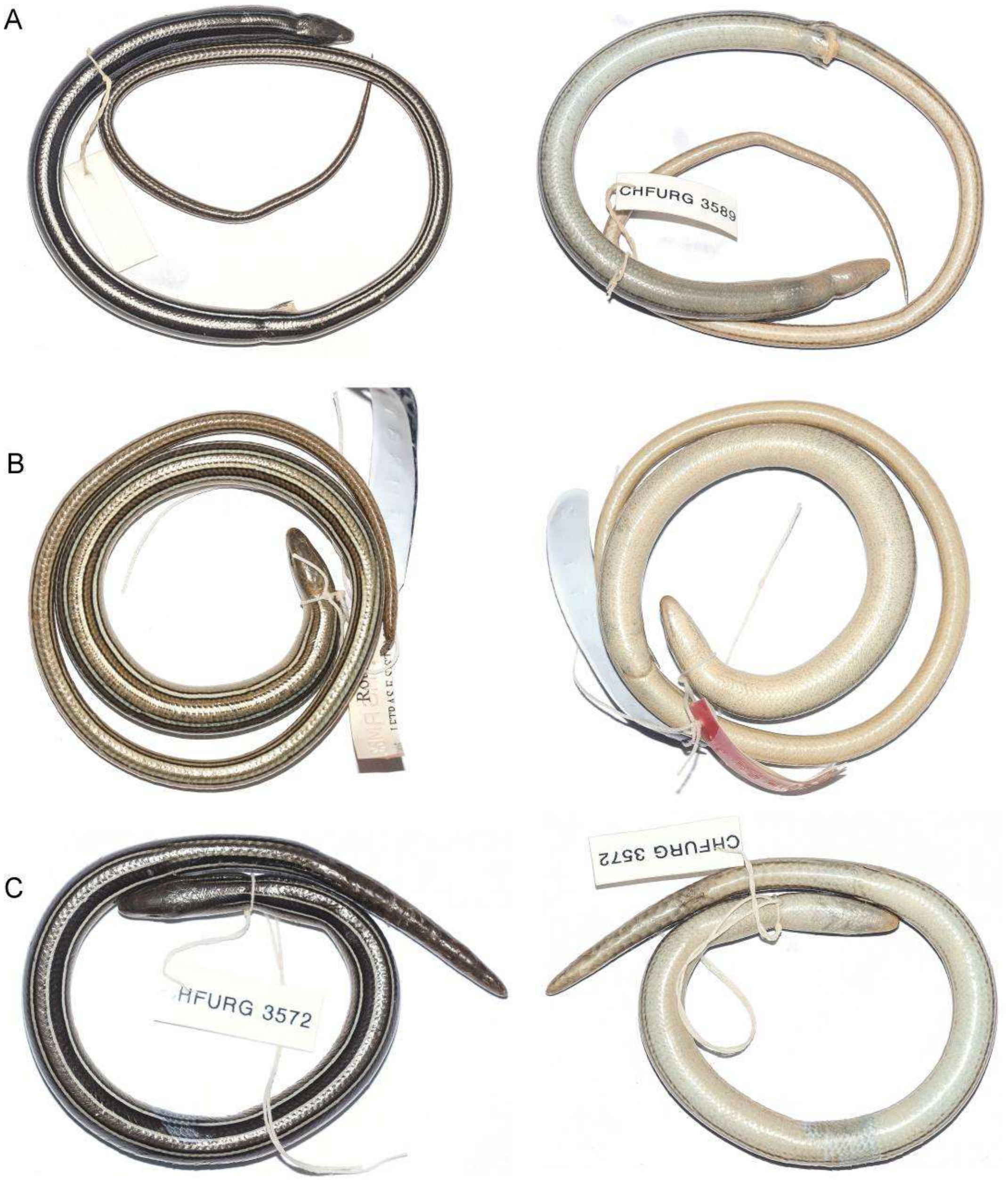

Fig. 2. Color variation of *O. enso* sp. n. (A – Holotype, CHFURG 3589; B – Paratype, CHFURG 3588; C – Paratype, CHFURG 3572).

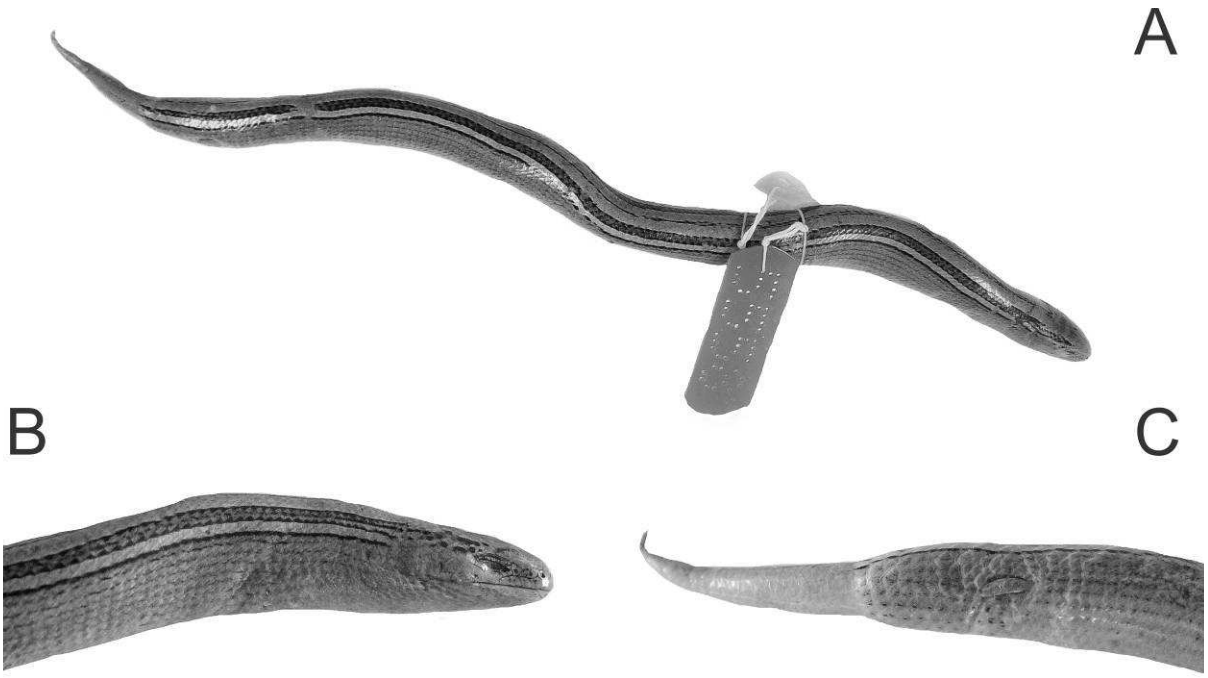

Fig. 3. Syntype of *Ophiodes vertebralis* (MNHN 5558; A – Dorsal pattern, B – Lateral head view, C – Hind limb view). Photo credits: Nicolas Vidal (MNHN).

Fig. 4. Known species of *Ophiodes*: (A – *O. vertebralis*, B – *O. enso* sp. n., C – *O. intermedius*, D – *O. striatus*, E – *O. luciae*, F – *O. fragilis*).

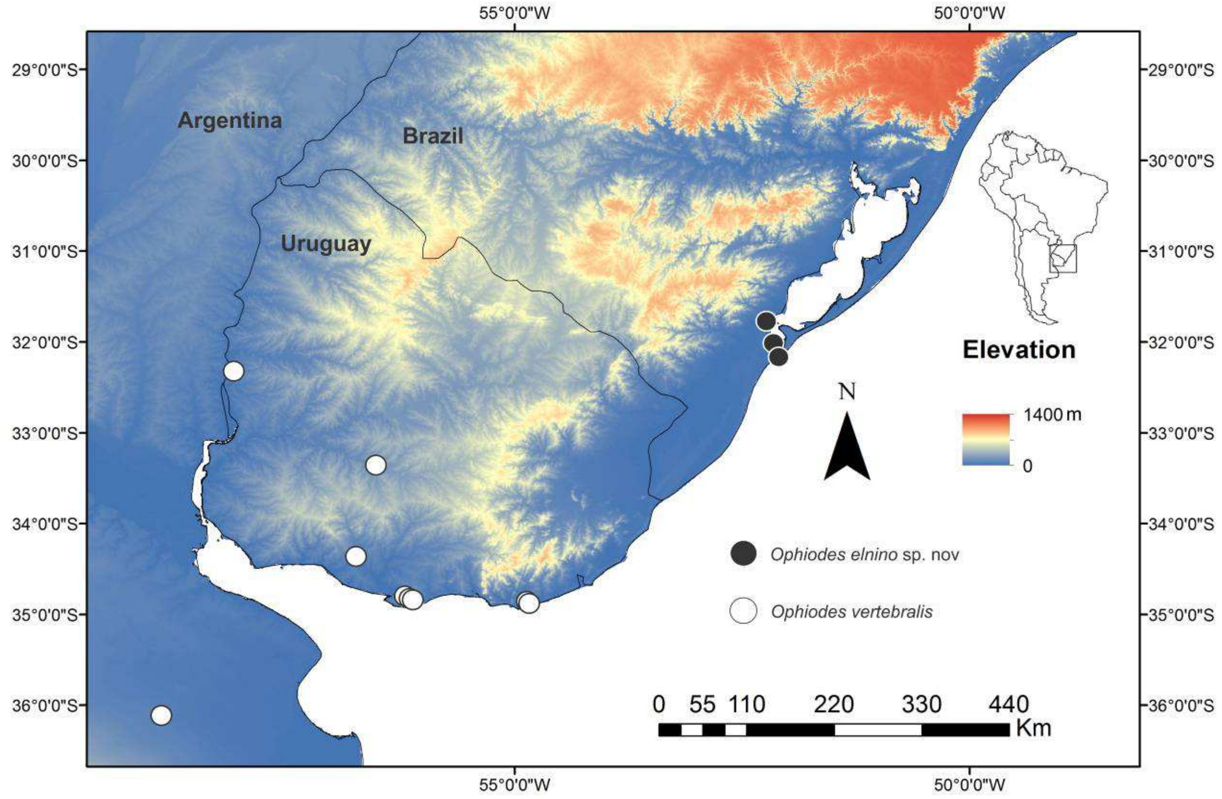

Fig. 5. Geographical distribution of *O. enso* sp. n. (black circles) and *O. vertebralis* (white circles) I eastern South America.

APÊNDICE 4

Manuscrito formatado e submetido para o periódico Brazilian Journal of Biology

Título: Electrocuted! Amphibian deaths caused by electric discharge

Autoria: Ruth Anastasia Regnet e Daniel Loebmann

Número de palavras: 896

Figuras: 1

Qualis Capes 2017: B3

Fator de impacto: 0,783

Electrocuted! Amphibian deaths caused by electric discharge

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1 figure

Running title: Amphibian deaths caused by electric discharge

In the past decades, a relatively large number of researchers have mapped the causes accounting for the decline of several amphibian species, a known global phenomenon since the early 1990s (Wake, 1991). Although habitat loss and overutilization have been pinpointed as the main motivators of Amphibians declines (e.g. Stuart et al., 2004; Becker et al., 2007; Eigenbrod et al., 2008; Harper et al., 2008; Smith et al., 2009), others biological and anthropic factors have directly impacted amphibian communities (see Alford & Richards, 1999; Young et al., 2001; Hayes et al., 2010). In a local-regional perspective, factors such as the use of road salt, catastrophic events, fires, among others, are responsible for population declines. Herein, we described an episode of anuran deaths caused by eletric discharge in a temporary pond.

On 07 November 2016 at 8:30 hrs, we found a group of 15 dead mature frogs in a temporary pond located at Universidade Federal do Rio Grande (FURG), municipality of Rio Grande, state of Rio Grande do Sul, Brazil (-32.0754°,S -52.1689, 7 m above sea level). All of them were found in a ca. 2 m radius from a lamppost which was flooded on its basis (Fig. 1). We used a Minipa Et- 3110 Digital Clamp Multimeter to measure voltage and recorded a current of 34.8 v in the water. The following species were recorded dead: *Elachistocleis bicolor* (Guérin-Méneville, 1838) (2 males and 2 females), *Leptodactylus latrans* (Steffen, 1815) (1 male and 5 females), one male of *Pseudopaludicola falcipes* (Hensel, 1867), and one male of *Rhinella arenarum* (Hensel, 1867). A total of three individuals were note possible to identify to the species level due to the high level of putrefation. Snout-vent length among species ranged from 15.3 mm to 87.7 mm. The number of deaths by electric discharge was underestimated, seeing as some individuals were consumed by native birds (RAR personal observation). Dead specimens were collected and deposited at the Herpetological Collection of FURG, collecting numbers 5258 to 5269.

Our findings revealed anuran mortality caused by dissipated electricity in the water. Although this situation seems to be unusual, power grids installed in temporary pond sites may dissipate electricity through the water. Our concern regarding this subject is the synchronism between the formation of temporary ponds and anuran aggregation for reproduction. Such a confluence of events should be highlighted in our study area, since it is the preferred breeding habitat for most species (Loebmann, 2005). Additionally, this scenario may impact tadpoles that develop at that same pond.

Hayes et al. (2010) summarized a collection of causes responsible for amphibian declines and split them into three distinct levels. These causes may act isolated or combined, including vertical interactions between levels. Following Hayes' classification, our findings can be classified as Level 2 factors (factors contributing to death and failed recruitment), factor 'other mortality' (i.e. caused by incidental deaths in the current report). There are no previous studies reporting amphibian mortality caused by incidental electric discharge. This fatality emphasizes the consequences of the loss of natural habitat by amphibians and, therefore, reinforces the necessity to properly manage the natural dynamism of temporary lakes, protecting aquatic biota from anthropic impacts.

RAR thanks CAPES for providing the MSc. scholarship. Authors are grateful to staff of the electronic department of FURG, who helped measure electric currents at the pond.

Literature Cited

ALFORD, R.S. and RICHARDS, S.J., 1999. Global amphibian declines: A problem in Applied Ecology. *Annual Review of Ecology and Systematics*, vol. 30, pp. 133-165.

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

EIGENBROD, F., HECNAR, S.J. and FAHRIG, L., 2008. Accessible habitat: an improved measure of the effects of habitat loss and roads on wildlife populations. *Landscape Ecology*, vol. 23, pp. 159-168.

HARPER, E.B., RITTENHOUSE, T.A.G. and SEMLITSCH, R.D., 2008. Demographic consequences of terrestrial habitat loss for pool-breeding amphibians: predicting extinction risks associated with inadequate size of buffer zones. *Conservation Biology*, vol. 22, pp. 1205-1215.

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

LOEBMANN, D., 2005. *Guia Ilustrado: Os anfíbios da região costeira do extremo sul do Brasil.* 1st ed. Pelotas: União Sul-Americana de Estudos da Biodiversidade, 76 p.

SMITH, K.G., LIPS, K.R., and CHASE, J.M., 2009. Selecting for extinction: nonrandom disease-associated extinction homogenizes amphibian biotas. *Ecology Letters*, vol. 12, pp. 1069-1078.

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

WAKE, D.B., 1991. Declining amphibian populations. *Science*, vol. 253, pp. 860.

YOUNG, B.E., LIPS, K.R., REASER, J.K., IBANEZ, R., SALAS, A.W., CEDENO, J.R., COLOMA, L.A., RON, S., LA MARCA, E., MEYER, J.R., MUNOZ, A., BOLANOS, F., CHAVES, G., and ROMO, D. 2001. Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology*, vol. 15, pp. 1213-1223.

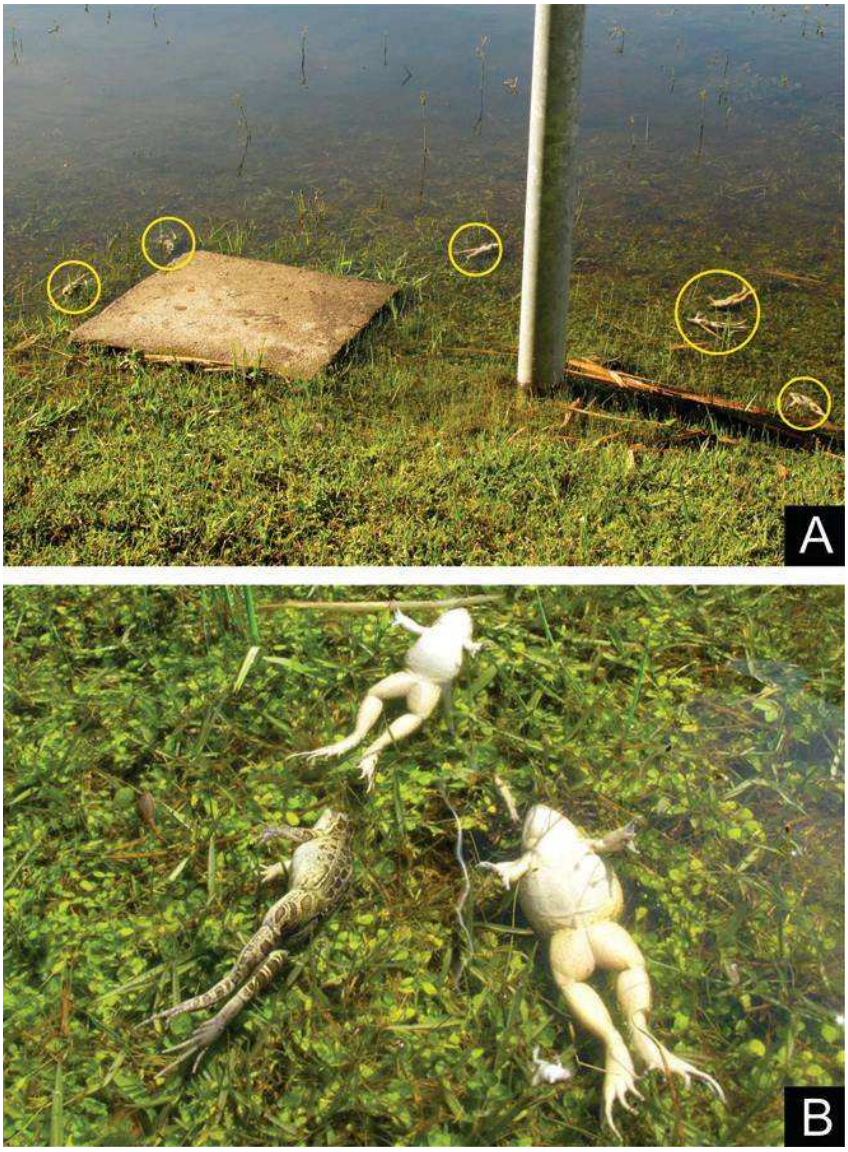

Figure 1. A – Lamppost which was flooded on its basis with dead specimens highlighted in yellow circles; B - Some of the dead individuals in closeup. Photograph by Ruth A. Regnet.