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

Luiza Loebens

CICLO REPRODUTIVO DE *PHILODRYAS PATAGONIENSIS* (SERPENTES: DIPSADIDAE) NO SUL DO BRASIL

Santa Maria, RS
2015

Luiza Loebens

CICLO REPRODUTIVO DE *PHILODRYAS PATAGONIENSIS* (SERPENTES: DIPSADIDAE) NO SUL DO BRASIL

Dissertação de Mestrado apresentada ao Curso de Pós-Graduação em Biodiversidade Animal da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciências Biológicas - Área Biodiversidade Animal**

Orientadora: Profª. Drª. Sonia Zanini Cechin
Coorientadora: Drª. Selma Maria Almeida-Santos

Santa Maria, RS
2015

LUIZA LOEBENS

CICLO REPRODUTIVO DE *PHILODRYAS PATAGONIENSIS* (SERPENTES: DIPSADIDAE) NO SUL DO BRASIL

Dissertação de Mestrado apresentada ao Curso de Pós-Graduação em Biodiversidade Animal da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciências Biológicas - Área Biodiversidade Animal**

Aprovado em 07 de dezembro de 2015:

_____
Sonia Zanini Cechin, Dr^a.
(Presidente/Orientadora)

_____
Claudio Augusto Rojas, Dr. (UNIFESP)

_____
Paulo Afonso Hartmann, Dr. (UFFS)

Santa Maria, RS
2015

AGRADECIMENTO

RESUMO

CICLO REPRODUTIVO DE *PHILODRYAS PATAGONIENSIS* (SERPENTES: DIPSADIDAE) NO SUL DO BRASIL

AUTORA: LUIZA LOEBENS
ORIENTADORA: SONIA ZANINI CECHIN
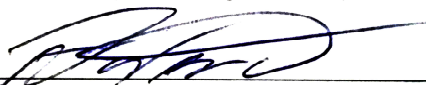CO-ORIENTADORA: SELMA MARIA ALMEIDA-SANTOS

Serpentes tropicais apresentam uma grande diversidade de padrões reprodutivos influenciados por fatores intrínsecos e condições ambientais. Ciclos reprodutivos podem apresentar maior plasticidade em áreas tropicais, devido à complexidade climática. No entanto, estudos de biologia reprodutiva de serpentes são mais desenvolvidos com espécies de regiões temperadas. Neste trabalho investigamos a biologia reprodutiva da serpente "papa-pinto" (*Philodryas patagoniensis*) através das análises morfológicas e histológicas de espécimes do sul do Brasil. Para isso, foram avaliados indivíduos depositados em coleções zoológicas para análise dos testículos, ductos deferentes e rins nos machos, assim como ovários e ovidutos nas fêmeas. O pico espermatogênico coincide com a época de acasalamento, no entanto é possível observar esperma no ducto deferente ao longo de todo o ano. As fêmeas permanecem reprodutivamente ativas durante um período estendido apresentando folículos vitelogênicos em todas as estações do ano, o pico de vitelogênese ocorre no período o inverno-primavera. As fêmeas têm capacidade para produzir múltiplas desovas na mesma estação reprodutiva com o esperma armazenado a longo prazo. O dimorfismo sexual do tamanho do corpo é evidenciando, sendo as fêmeas significativamente maiores e mais pesadas, os machos possuem caudas maiores do que as fêmeas. Os machos atingem a maturidade sexual em tamanhos menores do que as fêmeas. A fecundidade em *P. patagoniensis* está correlacionada com o tamanho corpóreo materno, com fêmeas maiores produzindo folículos e ovos em maior quantidade e tamanho. O número de ovos produzidos por cada fêmea varia entre cinco e 22. A atividade sazonal em *P. patagoniensis* é influenciada pelo ciclo reprodutivo, uma vez a época de acasalamento nos adultos (primavera) e recrutamento nos juvenis (verão) coincide com o pico de atividade. As mudanças sazonais na temperatura são o principal fator que influencia a atividade reprodutiva das serpentes em clima subtropical. De forma geral, nossos resultados indicam que o *P. patagoniensis* tem um padrão sazonal da reprodução no sul do Brasil.

Palavras-chave: reprodução; espermatogênese; vitelogênese; estocagem de esperma; desovas múltiplas; dimorfismo sexual.

ABSTRACT

Master Dissertation
Post-Graduation in Animal Biodiversity
Santa Maria Federal University

PHILODRYAS PATAGONIENSIS (SERPENTES: DIPSADIDAE) REPRODUCTIVE CYCLE IN SOUTHERN BRAZIL
AUTHOR: LUIZA LOEBENS
ADVISOR: SONIA ZANINI CECHIN
CO-ADVISOR: SELMA MARIA ALMEIDA-SANTOS

Tropical snakes present a greater diversity of reproductive patterns influenced by intrinsic factors and environmental conditions. Reproductive cycles may be more plastic in tropical areas due to the climatic complexity. However, studies of snake reproductive biology are more developed with species by temperate region. So, we investigated the reproductive biology of the snake Patagonia Green Racer (*Philodryas patagoniensis*) through anatomical and histological analysis of specimens from south Brazil. For that, individuals deposited in zoological collections were used to analyze the males testes, ductus deferens, and kidney, and the females ovarium and oviducts. The spermatogenic peak coincides with the mating season, although sperm was seen stored in ductus deferens all over the year. The females stay reproductively active during an extended period showing vitellogenic follicles in all the seasons, beside that the vitellogenic peak occurs during winter-spring. Females have ability to produce multiple clutches in the same reproductive season with sperm long-term stored. Sexual body size dimorphism was evident being females significantly larger and heavier than males, beside that males have larger tails than females. Males the sexual maturity in smaller sizes than females. Fecundity in *P. patagoniensis* is correlated with maternal body size, with larger females producing follicles and eggs in greater amount and size. The number of eggs produced by each female varies between five and 22. The seasonal activity is determined by the reproductive cycle, since males and females shows activity peak (spring) coinciding with the mating season, and juveniles were more abundant in the time of recruitment (summer). The seasonal changes in temperature must be the main factor influencing the snakes reproductive activity in subtropical climate. Altogether, our results indicate that *P. patagoniensis* has a seasonal pattern of reproduction in south Brazil.

Keywords: reproduction; spermatogenesis; vitellogenesis; sperm storage; multiple clutches; sexual dimorphism.

SUMÁRIO

INTRODUÇÃO

A reprodução é um aspecto fundamental da história de vida dos organismos, afinal relaciona-se diretamente com a aptidão individual. Estudos sobre estratégias reprodutivas fornecem rico material sobre a adaptação das histórias de vida às condições locais (VITT & CALDWELL, 2014). Sendo que alguns autores consideram as serpentes organismos modelo em estudos de biologia reprodutiva (SHINE & BONNET, 2000; BLACKBURN, 2006).

Estratégias reprodutivas dizem respeito a caracteres morfológicos, fisiológicos e comportamentais apresentados por uma espécie a fim de garantir o sucesso reprodutivo. Como estratégias reprodutivas podemos citar: investimento e esforço reprodutivo, ciclo gametogênico, modo reprodutivo, sincronia reprodutiva populacional, período de cópula, rituais de acasalamento, frequência reprodutiva e cuidado parental (SEIGEL & FORD, 1987; SHINE, 2003). A evolução de uma determinada estratégia reprodutiva está relacionada a forma como uma espécie consegue lidar com as restrições ambientais afim de garantir o sucesso reprodutivo (MADSEN & SHINE, 1999). Nesse contexto, a ordem dos escamados (Squamata) exibe ampla diversidade de estratégias reprodutivas, que excedem o simples impasse oviparidade-viviparidade (SHINE, 1983; ANDREWS & MATHIES, 2000).

A reprodução em serpentes é comumente analisada a fim de se estabelecer um padrão, que permita realizar comparações entre as espécies por meio de uma classificação do ciclo (SHINE, 2003). As classificações clássicas levam em consideração a relação entre a época de cópula e a produção de gametas, sendo o ciclo: pós-nupcial (dissociado), se a época de acasalamento ocorre quando as gônadas estão quiescentes e os níveis de hormônios sexuais circulantes são baixos; ou pré-nupcial (associado), quando a época de acasalamento coincide com o período de máxima atividade das gônadas e os níveis de esteroides encontram-se elevados (VOLSØE, 1944; SAINT GIRONS, 1982; CREWS, 1984; SEIGEL & FORD, 1987). Quando ocorre ciclo pós-nupcial, a cópula pode ocorrer em época distinta da fecundação. Neste caso, os espermatozoides são estocados na época de acasalamento e permanecem no trato genital das fêmeas até o momento da ovulação (SCHUETT, 1992). Já em outras situações, os machos podem acasalar com fêmeas apesar de apresentarem testículos inativos, pois são capazes de armazenar gametas nos ductos deferentes (ALMEIDA-SANTOS et al., 2004).

Outra forma de classificação clássica leva em consideração a extensão do período de produção de gametas, sendo o ciclo contínuo (assazonal), quando a produção de gametas ocorre ao longo de todo o ano, ou descontínuo (sazonal), quando os gametas são produzidos em uma

época restrita (SAINT GIRONS, 1982). Contudo, sabe-se que um ciclo verdadeiramente contínuo é praticamente inexistente em serpentes (ALMEIDA-SANTOS et al., 2006).

A classificação mais recente considera que a ciclicidade reprodutiva e sua expressão temporal nos indivíduos de ambos os sexos produz a ciclicidade observável em nível populacional. Assim, o ciclo reprodutivo em nível de indivíduo pode ser classificado em: (1) descontínuo, se as gônadas e órgãos sexuais acessórios permanecem inativos durante algum período do ano; (2) contínuo, se as gônadas e órgãos sexuais acessórios não se tornam completamente inativas, mas apresentam atividade reduzida por algum período do ano; ou (3) acíclico, se as gônadas e órgãos sexuais acessórios apresentam níveis essencialmente constantes de atividade ao longo do ano. Enquanto em nível de população, o ciclo reprodutivo pode ser: (1) sazonal, se um número proporcional de indivíduos da população exibe estágios reprodutivos semelhantes em uma determinada época restrita do ano, de modo que haja alguma sincronia na gametogênese entre os indivíduos; ou (2) assazonal, se existem proporções uniformes de indivíduos em estágios reprodutivos semelhantes em todos os meses do ano, sem que exista uma sincronia na gametogênese entre os indivíduos (MATHIES, 2011).

O ciclo reprodutivo de uma espécie de serpentes relaciona-se diretamente a fatores extrínsecos, como fatores climáticos e disponibilidade de recursos (BROWN & SHINE, 2006). Por outro lado, grande parte das estratégias reprodutivas devem-se a fatores intrínsecos, uma vez que se conservam filogeneticamente (BARROS et al., 2014), e certo grau de variação é consentido em função da plasticidade fenotípica (SEIGEL & FORD, 1991). Nas regiões tropicais e subtropicais, os ciclos reprodutivos tendem ser a mais diversificados (SEIGEL & FORD, 1987). Entretanto, estudos reprodutivos são historicamente mais desenvolvidos com serpentes de zonas temperadas (PIZZATTO et al., 2008).

Entre as famílias de serpentes neotropicais, a Dipsadidae apresenta maior diversidade, com aproximadamente 65% das espécies descritas (BÉRNILS & COSTA, 2012). As espécies pertencentes a essa família estavam até recentemente alocadas na família Colubridae, mas análises filogenéticas moleculares reclassificaram as serpentes superiores (Caenophidia) dividindo os colubrídeos em duas famílias: Colubridae e Dipsadidae (ZAHER et al., 2009).

Dentro da família Dipsadidae, subfamília Xenodontinae está o gênero *Philodryas,* com 22 espécies, todas restritas da América do Sul, das quais oito ocorrem na região Sul do Brasil (UETZ, 2015). Dentre as espécies do gênero *Philodrya*s que ocorrem na região Sul, apenas quatro tiveram seu ciclo reprodutivo estudado: *P. aestiva* (FOWLER et al., 1998), *P. agassizi* (MARQUES et al., 2006), *P. olfersii* (FOWLER et al., 1998; MESQUITA et al., 2013) e *P. patagoniensis (*FOWLER et al., 1998; PONTES, 2007*).* Vale ressaltar que os estudos citados

dão maior ênfase ao ciclo reprodutivo das fêmeas e nenhum apresenta análise microscópica dos órgãos reprodutivos.

A serpente *Philodryas patagoniensis* (Girard, 1858) conhecida popularmente como papa-pinto ou parelheira, é uma espécie ovípara de coloração verde-oliva, porte mediano e hábito predominantemente terrestre (LÓPEZ & GIRAUDO, 2008). Ocorre principalmente em áreas abertas como savanas e campos, possuindo ampla distribuição no Brasil, Bolívia, Paraguai, Argentina e Uruguai (PETERS & OREJAS-MIRANDA, 1970).

É uma espécie notavelmente abundante na fauna neotropical e apresenta hábitos alimentares generalistas, consumindo principalmente vertebrados de pequeno porte como peixes, anuros, lagartos, serpentes, aves e roedores (HARTMANN & MARQUES, 2005; PONTES, 2007).

O ciclo reprodutivo de *P. patagoniensis* foi estudado por alguns autores que se utilizaram principalmente de dados morfológicos para suas análises. O ciclo reprodutivo das fêmeas e o dimorfismo sexual da espécie na região Sudeste do Brasil foram estudados por FOWLER et al. (1998) e FOWLER & SALOMÃO (1994), respectivamente. ROJAS et al. (2015) descreveu ainda o trato reprodutivo das fêmeas e estruturas envolvidas na estocagem de espermatozoides para a espécie no Sudeste. Padrões de estocagem de esperma e variações cíclicas ovidutais das fêmeas foram analisados de forma comparativa para as populações do Sul e Sudeste do Brasil (ROJAS, 2013). No Estado do Paraná, OLIVEIRA (1999) descreveu aspectos reprodutivos da espécie. Numa população do litoral norte do Rio Grande do Sul, PONTES (2007) analisou dimorfismo sexual, fecundidade, maturidade sexual e ciclo reprodutivo das fêmeas. Outro estudo realizado por LÓPEZ & GIRAUDO (2008) no Nordeste da Argentina descreve o dimorfismo sexual e ciclo reprodutivo das fêmeas.

Até o momento, nenhum estudo utilizou de análise histológica para descrever o ciclo reprodutivo de *P. patagoniensis*. Essa condição possivelmente ocorre devido à maior importância atribuída à fêmea no processo reprodutivo, sendo fácil avaliar a sua condição reprodutiva macroscopicamente pela presença de folículos e/ou ovos (ALMEIDA-SANTOS et al., 2014). Em contrapartida, uma compreensão mais profunda do ciclo reprodutivo requer a avaliação microscópica das gônadas e órgãos reprodutivos acessórios de machos e fêmeas (MATHIES, 2011).

Desta forma, neste trabalho é descrito o ciclo reprodutivo de *P. patagoniensis* no Sul do Brasil utilizando-se de técnicas morfológicas e histológicas. A presente dissertação é composta por dois artigos acerca da biologia reprodutiva de *P. patagoniensis*, a saber:

- Artigo 1: fornece a descrição do ciclo reprodutivo dos machos de *P. patagoniensis*. A biologia reprodutiva de machos foi analisada em termos de ciclo testicular, ciclo do segmento sexual renal, maturidade sexual, esforço reprodutivo e fecundidade (capacidade reprodutiva).

- Artigo 2: fornece a descrição do ciclo reprodutivo das fêmeas de *P. patagoniensis*. A biologia reprodutiva foi analisada em termos de ciclo ovariano, maturidade sexual, esforço reprodutivo e fecundidade.

Os artigos científicos estão formatados de acordo com as normas da Revista Acta Zoologica (ISSN 1463-6395).

REFERÊNCIAS

ALMEIDA-SANTOS, S. M.; LAPORTA-FERREIRA, I. L.; ANTONIAZZI, M. M. & JARED, C. Sperm storage in males of the snake *Crotalus durissus terrificus* (Crotalinae: Viperidae) in southeastern Brazil. **Comparative Biochemistry and Physiology**, Part A, v. 139, p. 169-174, 2004.

ALMEIDA-SANTOS, S. M.; PIZZATTO L. & MARQUES, O. A. V. Intra-sex synchrony and inter-sex coordination in the reproductive timing of the atlantic coral snake *Micrurus corallinus* (elapidae) in brazil. **Herpetological Journal**, v. 16, p. 371-376, 2006.

ALMEIDA-SANTOS, S. M.; BRAZ, H. B.; SANTOS, L. C.; SUEIRO, L. R.; BARROS, V. A.; ROJAS, C. A. & KASPEROVICZUS, K. N. Biologia reprodutiva de serpentes: recomendações para a coleta e análise de dados. **Herpetologia Brasileira**, v. 3, n. 1, p. 14-24, 2014.

ANDREWS, R. & MATHIES, T. Natural history of reptilian development: constraints on the evolution of viviparity. **Bioscience**, v. 50, n. 3, p. 227-238, 2000.

BARROS, V. A.; ROJAS, C. A. & ALMEIDA-SANTOS, S. M. Is rainfall seasonality important for reproductive strategies in viviparous neotropical pit vipers? A case study with *Bothrops leucurus* from the Brazilian Atlantic Forest. **Herpetological Journal**, v. 24, p. 69-77, 2014.

BÉRNILS, R. S. & COSTA, H. C. (Org.). Répteis brasileiros: Lista de espécies. Versão 2012.1. **Sociedade Brasileira de Herpetologia**, 2012. Disponível em: <http://www.sbherpetologia.org.br/>.Acesso em: 18 mar. 2014.

BLACKBURN, D. G. Squamate reptiles as model organisms for the evolution of viviparity. **Herpetological Monographs**, v. 20, p. 131-146, 2006.

BROWN, G. P. & SHINE, R. Why do most tropical animals reproduce seasonally? Testing hypotheses on an Australian snake. **Ecology,** v. 87, p. 133-143, 2006.

CREWS, D. Gamete production, sex hormone secretion, and mating behavior uncoupled. **Hormonal Behavior**, v. 18, p. 22-28, 1984.

FOWLER, I. R. & SALOMÃO, M. G. A study of sexual dimorphism in six species from the colubrid snake genus *Philodryas*. **The Snake,** v. 26, p. 117-122, 1994.

FOWLER, I. R.; SALOMÃO, M. G. & JORDÃO, R. S. A description of the female reproductive cycle in four species from the neotropical colubrid snake *Philodryas* (Colubridae. Xenodontinae). **The Snake,** v. 28, p. 71-78, 1998.

HARTMANN, P. A. & MARQUES, O. A. V. Diet and habitat use of two sympatric species of *Philodryas* (Colubridae) in south Brazil. **Amphibia-Reptilia,** v. 26, p. 25-31, 2005.

LÓPEZ, M. S. & GIRAUDO, A. R. Ecology of the Snake *Philodryas patagoniensis* (Serpentes, Colubridae) from Northeast Argentina. **Journal of Herpetology,** v. 42, n.3, p. 474-480, 2008.

MADSEN, T. & SHINE, R. The adjustment of reproductive threshold to prey abundance in a capital breeder. **Journal of Animal Ecology**, v. 68, p. 571-580, 1999.

MARQUES, O. A. V.; STENDER-OLIVEIRA, F.; SAWAYA, R. J. & FRANÇA, F. G. R. Ecology of the colubrid snake *Pseudablabes agassizii* in south-eastern South America. **Herpetological Journal**, v. 16, p. 37-45, 2006.

MATHIES, T. Reproductive Cycles of Tropical Snakes. In: ALDRIDGE, R. D.; SEVER, D. M. (Org.). **Reproductive Biology and Phylogeny of Snake**s. Science Publishers, 2011. (Coleção Reproductive Biology and Phylogeny, 9). cap. 12, p. 511-550.

MESQUITA, P. C. M. D.; SÁ-POLIDORO, G. L. & CECHIN. S. Z. Reproductive biology of *Philodryas olfersii* (Serpentes, Dipsadidae) in a subtropical region of Brazil. **Herpetological Journal,** v.23, p. 39-44, 2013.

OLIVEIRA, F. S. **Dieta e aspectos reprodutivos de *Philodryas patagoniensis* (Serpentes: Colubridae) no Estado do Paraná, Brasil.** 1999. 44 p. Monografia. (Bacharelado em Ciências Biológicas) - Universidade Federal do Paraná, Curitiba, PR, 1999.

PETERS, J. A. & B. OREJAS-MIRANDA. **Catalogue of the neotropical Squamata.** Part I - Snakes. United States National Museum Bulletin, v. 297, p. 1-347, 1970.

PIZZATTO, L.; CANTOR, M.; OLIVEIRA, J. L.; MARQUES, O. A. V.; CAPOVILLA, V. & MARTINS, M. Reproductive ecology of dipsadine snakes, with emphasis on South American species. **Herpetologica,** v. 64, 168-179, 2008.

PONTES, G. M. F. **História natural de *Philodryas patagoniensis* (Serpentes: Colubridae) no litoral do Rio Grande do Sul, Brasil**. 2007. 83 p. Tese (Doutorado em Zoologia) - Faculdade de Biociências. Pontífica Universidade católica do Rio Grande do Sul, Porto Alegre, RS, 2007.

ROJAS, C. A. **Padrões de estocagem de esperma e variações cíclicas ovidutais em serpentes Xenodontinae.** 2013. 173 p. Tese. (Doutorado Anatomia dos Animais Domésticos e Silvestres) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, SP, 2013.

ROJAS, C. A.; BARROS, A. V. & AMEIDA-13SANTOS, S. M. Sperm storage and morphofunctional bases of the female reproductive tract of the snake *Philodryas patagoniensis* from southeastern Brazil. **Zoomorphology,** v. 134, p 1-10, 2015.

SAINT GIRONS, H. Reproductive cycles of male snakes and their relationships with climate and female reproductive cycles. **Herpetologica**, v. 38, p. 5-16, 1982.

SCHUETT, G. W. Is long-term sperm storage an important component of the reproductive biology of temperate pitvipers? In: CAMPBELL, J. A. & BRODIE, E. D. (Eds.). **Biology of Pitvipers**. Selva, Texas, 1992. p. 169-184.

SEIGEL, R. A. & FORD, N. Reproductive ecology. In: SEIGEL, R. A.; COLLINS, J. T.; NOVAK, S. S. (Eds.) **Snakes, Ecology and Evolutionary Biology**. McMillan Publishing Company, New York, 1987. cap. 8, p. 210-252.

SEIGEL, R. A. & FORD, N. B. Phenotypic plasticity in the reproductive characteristics of an oviparous snake, *Elaphe guttata*: Implications for life history studies. **Herpetologica.** v. 47, p. 301-307, 1991.

SHINE, R. Reptilian reproductive modes: The oviparity-viviparity continuum. **Herpetologica**, v. 39, n. 1, p. 1-8, 1983.

SHINE R. Reproductive strategies in snakes. **Proceedings of the Royal Society B: Biological Sciences**, v. 270, p. 995-1004, 2003.

SHINE, R. & BONNET, X. Snakes: a new "model" organism in ecological research? **Trends in Ecology and Evolution.** v. 15, p. 221-222, 2000.

UETZ, P. 2015. **Reptile Database.** Disponível em: <http://reptile-database.reptarium. cz/advanced_search?genus=philodryas&submit=Search>. Acessado em: 13 nov. 2015.

VITT, L. J. & CALDWELL, J. P. **Herpetology: An Introductory Biology of Amphibians and Reptiles**. 4. ed. Academic Press, San Diego. 734 pp, 2014.

VOLSØE, H. Seasonal variation of the male reproductive organs of *Vipera berus* (L.). **Spolia Zoologica Musei Hauniensis,** v. 5, p. 1-157, 1944.

ZAHER, H.; GRAZZIOTIN, F. G.; CADLE, J. E.; MURPHY, R. W.; MOURA-LEITE, J. C. & BONATTO, S. L. Molecular phylogeny of advanced snakes (Serpentes, Caenophidia) with an emphasis on South American Xenodontines: a revised classification and descriptions of new taxa. **Papéis Avulsos de Zoologia**, v. 11, p. 115-153, 2009.

1 REPRODUCTIVE BIOLOGY OF THE *PHILODRYAS PATAGONIENSIS* (SNAKES: DIPSADIDAE) IN SOUTH BRAZIL: MALE REPRODUCTIVE CYCLE

BIOLOGIA REPRODUTIVA DE *PHILODRYAS PATAGONIENSIS* (SERPENTES: DIPSADIDAE) NO SUL DO BRASIL: CICLO REPRODUTIVO DOS MACHOS

Luiza Loebens[1*], Sonia Zanini Cechin[1], Tiago Felipe Theis[1], Livia Bataioli Moura[1], Selma Maria Almeida-Santos[2]

ABSTRACT

The male reproductive cycle of Philodryas patagoniensis in south Brazil was described through morpho–anatomical and histological analysis of individuals deposited in zoological collections. Spermatogenesis occurred during late autumn–winter (Jun–Set) and spermiogenesis occurred in spring–summer (Oct–Mar). The volume of the testes was smaller (quiescent) in winter, while the tubular diameter and the epithelial height of the seminiferous tubule were larger in summer (Jan–Mar). The ductus deferens presented spermatozoa all over the year and had no seasonal variation in diameter. The length of the kidney was larger in winter–spring (Jul–Dec), although the tubular diameter and epithelium height of the sexual segment of the kidney (SSK) were larger only in winter (Jul–Sep). Total testicular regression was observed in late autumn (May), simultaneously with the peak in SSK. Therefore, at the individual level, males exhibit a discontinuous cyclical reproduction. Considering the population level, the reproductive cycle is seasonal semisynchronous, with most of the individuals showing a reproductive peak in spring–summer (Out–Mar). Here, we present evidence to support the importance of the microscopic approach to reproductive cycles studies. Finally, we discuss the intrinsic and extrinsic factors influencing *P. patagoniensis* reproductive patterns.

KEYWORDS: spermatogenesis; sexual segment of the kidney; ductus deferens; sperm storage.

[1] Laboratory of Herpetology, Santa Maria Federal University, Santa Maria, Rio Grande do Sul, Brazil

[2] Laboratory of Ecology and Evolution, Butantan Institute, São Paulo, São Paulo, Brazil

[*]Corresponding author: Laboratory of Herpetology, Santa Maria Federal University (UFSM), Santa Maria, 1000 Roraima Avenue, Camobi, Santa Maria, Rio Grande do Sul (RS) 97105-900, Brazil; e-mail address:loebens.luiza@gmail.com.

RESUMO

O ciclo reprodutivo de machos de *Philodryas patagoniensis* no Sul do Brasil é descrito através de análises morfoanatômicas e histológicas de indivíduos depositados em coleções zoológicas. A espermatogênese ocorre do final do outono até inverno (Jun-Set) e a espermiogênese ocorre no período primavera-verão (Out-Mar). O volume dos testículos foi menor (quiescente) no inverno, enquanto o diâmetro tubular e a altura do epitélio dos túbulos seminíferos foram maiores no verão (Jan-Mar). Os ductos deferentes apresentaram espermatozoides durante todo o ano e não demonstraram nenhuma variação sazonal no diâmetro. O comprimento do rim foi maior no período inverno-primavera (Jul-Dez), entretanto o diâmetro tubular e a altura do epitélio do segmento sexual renal (SSK) apresentaram-se aumentados apenas no inverno (Jul-Set). O estado de regressão total dos testículos foi observado no final do outono (Maio), simultaneamente ao pico do SSK. Então, em nível de indivíduo os machos exibem um ciclo reprodutivo descontínuo cíclico. Enquanto, a nível populacional, o ciclo reprodutivo é sazonal semi-sincrônico, com a maioria dos indivíduos apresentando pico reprodutivo no período primavera-verão (Out-Mar). Neste trabalho apresentamos evidências que suportam a teoria de que a análise histológica é fundamental para descrição precisa dos eventos reprodutivos. Por fim, discutimos fatores intrínsecos e extrínsecos que influenciam o padrão reprodutivo de *P. patagoniensis.*

PALAVRAS-CHAVE: espermatogênese; segmento sexual renal; ducto deferente; estocagem de esperma.

1.1 INTRODUCTION

Reproduction is a central aspect of the life history of organisms, and information on reproductive strategies of a substantial amount of snake taxa is essential to investigate the evolution of reproductive traits and its patterns (Almeida–Santos and Salomão 2002). Although, the reproduction is not crucial for the immediate survival, it is the currency of an individual's fitness, since it is essential for the species persistence. Nevertheless, reproduction is a critical event in the life of an individual because it represents a substantial energetic cost, especially to ectotherms animals which have low maintenance costs (Vitt and Caldwell 2014). Therefore, reproduction is not a continuous endeavor, and an appropriate timing of reproductive effort is critical (Brown and Shine 2006).

Reproductive cycles of snake populations vary from highly seasonal to aseasonal (Mathies 2011). While the majority of snakes from temperate zones present seasonal

reproduction, in tropical and subtropical snakes the cycles trend to be more plastic: species reproduce in the dry season, wet season, over extended periods, or even almost continuously (Seigel and Ford 1987). Summarizing, reproductive cycles of many snakes may vary over time, among populations, and even among individuals, being not easy to classify into discrete categories. Hence, the currently known diversity of reproductive patterns of snakes suggests that a single explanation on this matter is not enough.

In squamates, the male spermatogenetic cycle may or may not coincide with the female reproductive cycle. In this context, the reproductive cycle may be defined as associated when gonadal and hormonal events in males and females coincide with the mating season. However, in species that the sperm production is not synchronous with the females ovulation and fertilization, the cycle is defined as dissociated (Volsøe 1944; Saint Girons 1982; Seigel and Ford 1987; Aldridge *et al*. 2009). In these cases of desynchronization between the timing of spermatogenesis, ovulation, and mating, sperm storage appears to be obligatory (Sever and Hamlett 2002; Almeida–Santos *et al*. 2004). In male squamates, the ductus deferens evolved gradually through the phylogeny of this group as the main and long–term storage organ (Almeida–Santos *et al*. 2004; Sever 2004; Liang *et al*. 2011). Therefore, sperm storage is a reproductive strategy that established as a necessary stage in the reproductive cycle of squamates.

Furthermore, we should take account of the synchrony of reproductive cyclicality at the individual level and how this contributes to the seasonality of reproduction at the population level. For example, if individual males are seasonally reproductive, the male population cycle might be continuous if there are always a few males in spermatogenic condition in the population. In the opposite situation, if males in spermiogenesis condition occur in a restricted season of the year, the population cycle might be seasonal (Mathies 2011).

Another feature to be analyzed in the reproductive cycle of male squamates is the sexual segment of the kidney (SSK), a sexually dimorphic structure that has secretory activity under the control of testosterone (Krohmer, Martinez and Mason 2004; Aldridge *et al*. 2011; Rojas, Barros, and Almeida–Santos 2013). The SSK secretions are mixed with the semen and transmitted to the female during copulation (Aldridge *et al*. 2011), therefore several functions have been proposed for this secretion; it may compose the seminal fluid, nurturing and activating the sperm (Bishop 1959). It may prevent or reduce subsequent re–mating by the female (Nilson and Andrén 1982), and act in the formation of the copulatory plug to maintain sperm stored in the oviduct (Almeida–Santos and Salomão 1997; Almeida–Santos *et al*. 2004).

In addition, it may form a non–coagulated copulatory plug that acts as a viscous barrier to reduce the likelihood or speed of sperm transmission (Shine, Olsson and Mason 2000).

In this respect, until recently, the reproductive patterns of neotropical snakes were relatively less understood than those from temperate zones (Mathies 2011). Nevertheless, in the past two decades, Brazilian researchers have contributed considerably to the knowledge on the reproductive biology of neotropical snakes (Almeida–Santos *et al.* 2014). For a long time, reproductive data were only available for females (Barros and Almeida–Santos 2012), and several studies about male reproduction showed ambiguous results (Mathies 2011). However, recent researches had also included histological analysis to describe the spermatogenic and oviductal cycle of neotropical snakes (Rojas *et al.* 2013; Barros, and Almeida–Santos 2014*a, b*; Braz, Kasperoviczus and Almeida–Santos 2014; Resende and Nascimento 2015).

The *P. patagoniensis* (Girard 1858) is widely distributed in open areas of Brazil, Bolivia, Paraguay, Argentina, and Uruguay (Peters and Orejas–Miranda 1970). Available information on its reproductive biology concerns aspects of female reproductive cycle, sexual dimorphism, and oviductal sperm storage (Fowler and Salomão 1994; Fowler, Salomão and Jordão 1998; Pontes 2007; López and Giraudo 2008; Rojas, Barros and Almeida–Santos 2015). The species is oviparous, reproducing seasonally. Vitellogenesis occurs from August to February (autumn–summer); clutches were recorded from November to January (spring–summer) and births from January to March (summer–early autumn) (Pontes 2007; Lopéz and Giraudo 2008). Although direct evidence of the timing of mating (e.g. observations in the wild) is not available for *P. patagoniensis,* there is a single report of a probable mating in spring–summer (Sonia Z. Cechin 2014, pers. comm. 15 July).

Here we describe the males reproductive biology of the snake *P. patagoniensis* in the south region of Brazil. The aims of this study are: a) to describe the male reproductive cycle through morphological and histological analyses of the testes, SSK, and ductus deferens; and b) to investigate the size–fecundity relationships and the reproductive investment.

1.2 METHOD

Data collection

We analyzed 85 *P. patagoniensis* males from the south region of Brazil (Rio Grande do Sul, Paraná, and Santa Catarina States) available in the herpetological collections of the

Santa Maria Federal University (ZUFSM) and Pontifical Catholic University of Rio Grande do Sul (MCP–PUCRS). For each individual, we obtained information on month of death, snout–vent length (SVL), and body mass (BM).

The climate in the south region of Brazil is classified as humid subtropical (Köppen's climate classification Cfa-Cfb). The rainfall is well distributed throughout the seasons (mean annual precipitation 1.000–2.000 mm). However, the region shows well–defined temperature seasonality, with temperatures ranging from 0°C (winter) to 40°C (summer) (Alvares *et al.* 2013).

Reproductive events were described according to austral seasons. The reproductive cycle of mature males was analyzed considering morphological, macroscopic, and microscopic changes of the testes, ductus deferens, and kidney.

Macroscopic data

For each specimen, the following macroscopic data were collected: mass, length, width, and thickness of the testes, width of the distal portion of ductus deferens, and length and width of the proximal region of kidney (Rojas *et al.* 2013). Testicular volume (TV) was calculated by the ellipsoid formula: $V = (4/3) \pi abc$, where a = half of length, b = half of width, and c = half thickness of the testes (Pleguezuelos & Feriche 1999). The Gonadosomatic Index (GSI) was calculated by the formula according to: testes mass/body mass x 100 (Clesson *et al.* 2002).

Histology

We dissected 61 specimens in order to obtain histological samples of the proximal region of the testes, distal region of the ductus deferens, and proximal region of the kidney (Rojas *et al.* 2013). As a standard procedure, only right–side organs were used, preserving the contralateral. The tissue samples were processed for light microscopy by Historesin (Leica) method. Sections 2µm thick (Leica RM2245 microtome) were stained with hematoxylin–eosin. Slides were analyzed using a ZEISS Axio Scope.A1 microscope with Axiocam MRc 5. We obtained 10 measurements of the microscopic variables of each individual: seminiferous tubule diameter and epithelial height, Leydig cell nuclear diameter, and tubular diameter of SSK and epithelial height (Rojas *et al.* 2013). Only seminiferous and SSK tubules presenting circular form in the transverse section were considered to the morphometric analysis.

In order to determine the sexual maturity, we used the presence of spermatozoa in the testes or ductus deferens as the main criterion, and the convoluted or non–convoluted aspect of the ductus deferens as a secondary criterion (Shine 1977). We analyzed the spermatogenic cycle according to stages classification: 1) complete regression, 2) early recrudescence, 3) late recrudescence, 4) early spermiogenesis, 5) spermiogenesis, and 6) early regression (Goldberg & Parker 1975). The male reproductive cycle was classified at the individual and population level according to Mathies (2011). The cycle of the sexual segment of the kidney (SSK) was classified into stages: 0) SSK not hypertrophied, 1) SSK hypertrophied with a few granules, 2) SSK cytoplasm full of secretory granules, 3) SSK secretory granules apically in the cytoplasm, and 4) maximum density of SSK secretory granules (Krohmer *et al*. 2004).

Statistical analysis

The correlation between the macroscopic measurements and the body length (SVL) was tested by linear regression (Shine 1977). Therefore, analysis of covariance (ANCOVA) was employed to test the seasonal variation in testes volume, ductus deferens width, and kidney length and width, using SVL as the covariate. The GSI was determined by ANOVA and the significant results were analyzed by the Tukey's test. Aiming to investigate the seasonal variation in seminiferous tubule diameter and epithelial height, Leydig cell nuclear diameter and SSK tubular diameter and epithelial height were used in the analysis of variance (ANOVA). Post hoc tests (Tukey) were used to identify differences between seasons.

In order to explore the synchronism between SSK and spermatogenic cycles we performed a correlation analysis (Pearson's coefficient) using the TV vs. kidney length, seminiferous tubule diameter vs. SSK tubule diameter, and seminiferous epithelial height vs. SSK epithelial height.

To investigate size–fecundity relationships we performed linear regressions using only BM as the predictor variable, because SVL and BM were strongly correlated ($r = 0.80$, $p < 0.0001$). Therefore, we analyzed the relationship between the following variables: BM vs. TV, and BM vs. testicular mass. Correlations between body size (SVL) and the values of GSI were tested through the Pearson's correlation coefficient.

Statistical analyses were performed using Statistica version 10 (Statsoft 2011. All variables were tested for normality and homogeneity of variances prior to analysis, data were log–transformed when necessary. The plots were created on Sigmaplot 12 (Systat 2011).

1.3 RESULTS

Spermatogenic cycle

Seasonal variation in some macroscopic parameters of the male reproductive organs was statistical identifiable. Testes volume showed a decrease during winter ($F = 17.1645$, $p < 0.0001$, Fig. 1A) in comparison to summer, autumn, and spring ($p < 0.001$ for the three seasons, Table 1). The GSI was different between the seasons ($F = 11.5245$, $p < 0.0005$, Fig. 1B), with an increase in testicular mass during the spring and summer, in comparison to autumn ($p < 0.05$ for both seasons, Table 1), and winter ($p < 0.05$ and $p < 0.005$, Table 1). The ductus deferens width was larger in spring, although no significant differences were observed in this feature between the seasons ($F = 0.4387$, $p = 0.7287$, Table 1). Sperms in the ampulla ductus deferens were observed all over the year, but in autumn and winter the spermatozoa density was visually reduced (Fig. 2A), when compared with spring and summer (Fig. 2B).

Considering the microscopic data, the variation in the seminiferous tubules diameter was seasonal ($F = 4.6791$, $p = 0.0059$, Fig. 1C) and was larger in summer, compared with winter and spring ($p < 0.05$ and $p < 0.01$, respectively, Table 2). Accordantly, the variation in the seminiferous epithelium height was seasonal ($F = 5.0417$, $p = 0.0042$, Fig. 1D) and it increased in summer, when compared with winter and spring ($p < 0.01$ and $p < 0.05$, respectively, Table 2). Leydig cell nuclear diameter differed between seasons ($F = 5.5430$, $p = 0.0024$, Fig. 1E) and it was larger in spring than in summer, autumn, and winter ($p < 0.05$ for the three seasons, Table 2).

Therefore, different developmental stages were characterized by cellular changes in the testes of *P. patagoniensis* (Table 3). Complete regression of the testes (Stage I, Fig. 3A) occurred in autumn (May) and it was characterized by the presence of spermatogonia A and B and Sertoli cells only. Early recrudescence (Stage II, Fig. 3B) was observed in late autumn (June) and early winter (July), when proliferarion of spermatogonia, primary spermatocytes, and first meiosis cells began the growth of the epithelium. Late recrudescence (Stage III, Fig. 3C) occurred in spring (July–September), with predominance of primary spermatocytes and emergence of round spermatids. This was the phase of maximum cellular division (meiosis), thus the number of spermatogonia decreased substantially. Early spermiogenesis (Stage IV) lasted from spring to early summer (October–January), when a few spermatogonias and spermatocytes were seen, but spermatids were the most prevalent cell type in the epithelium. At this time, many of the round spermatids had advanced to the elongating spermatid stage. The

spermiogenesis (Stage V, Fig. 3D) occurred from spring (November) to early autumn (March), the epithelium was high and composed by late spermatids and many mature spermatozoa were released into the lumen (spermiation). Early regression (Stage VI) occurred in autumn (April–May), presenting epithelium atrophy and few spermatids and the remainder of spermatozoa in the lumen. At this point, spermatocytes and spermatids left overs underwent degeneration, so cellular debris were evidenced in the lumen.

SSK cycle

Macroscopic measurements of *P. patagoniensis* kidneys presented significant differences in length values between the seasons ($F = 8.2871$, $p = 0.0008$, Fig. 1F), but not in width values ($F = 0.9216$, $p = 0.4552$, Table 1). The kidney length was larger in winter than in summer and autumn ($p < 0.001$ for both seasons, Table 1), and larger in spring than in summer ($p < 0.05$, Table 1).

The tubular diameter of the SSK differed among the seasons ($F = 4.0781$, $p = 0.012$, Table 4, Fig. 1G) and it was larger in winter than in spring and summer ($p < 0.05$ for both seasons, Table 4). The epithelial height of the SSK exhibited a seasonal variation ($F = 3.7512$, $p = 0.0164$, Fig. 1H), with a significant increase during winter in relation to spring and summer ($p < 0.05$ for both season, Table 3).

Although macroscopic differences in the kidney size were hardly detected, histological investigation revealed clear seasonal variation in SSK activity. The histology of kidney showed that the SSK was not hypertrophied in late summer–early autumn, which was the period of regression (Stage 0, Fig. 4A). At this phase, the SSK lumen was narrow and with vacuoles on the medial region of the tubule. Autumn was the period of the start of the activity with a few granules weakly stained in the basal region of the cells (Stage 1).

In winter, the SSK was characterized by the presence of several granules spread throughout the cytoplasm of the cells, wider tubule lumen, and basal nuclei (Stage 2, Fig. 4B). Subsequently, on late winter–spring, SSK cells showed a large amount of granules in the cytoplasm and secretory vesicles apically positioned prepared to be released into the lumen of the SSK tubule (Stage 3; Fig. 4C)

The secretory phases occurred in the spring and extended to summer. At this period, the number of serous granules in the SSK cytoplasm increased, causing a hypertrophy in the epithelium and a decrease in luminal volume. Secretory granules were observed filling the apical and basal portions of the SSK cells and in the lumen of the tubule (Stage 4; Fig. 4D).

Secretory activity was reduced in late summer, when granules were seen aggregated only towards the apical end of the cells, when secretory phase was resumed again.

Comparison of SSK and spermatogenic cycles

The SSK demonstrated maximum activity in winter, preceding the spermatogenic peak (late spring–summer). Thus, the correlation analysis confirmed that SSK and spermatogenic cycles exhibited negative relationship between the following measurements: TV vs. kidney length (r = –0.80, p < 0.05, Fig. 5A), seminiferous tubule diameter vs. SSK tubule diameter (r = –0.82, p < 0.05, Fig. 5B), and seminiferous epithelial height vs. SSK epithelial height (r = –0.87, p < 0.05, Fig. 5C).

Size–fecundity relationships

Body mass had a positive and significant effect on TV (r = 0.79, p < 0.0001). BM had a significant relationship with TV (R^2 = 0.61, F = 95.71, p < 0.0001), but not with testicular mass (R^2 = –0.011, F = 0.35, p = 0.56, Fig. 6A). The overall mean of the GSI for males was 1.13 ± 0.22 (range 0.11–3.8). The male SVL and reproductive effort (GSI) were positively correlated (r = 0.30, p < 0.05, Fig. 6B). The smallest mature male presented 61.1 g of mass, SVL of 440 mm, and total length of 690 mm.

1.4 DISCUSSION

P. patagoniensis exhibits spatial germ cell development of the testes, which is a common strategy seen within most of the amniotes (Granados-González *et al.* 2015) In this kind of gamete production, the germ cells populations are seen layered together in the seminiferous epithelium and–progress uniformly through the phases of spermatogenesis as a single cohort (Gribbins 2011).

The spermatogenic cycle of *P. patagoniensis* was extended but not continuous throughout the year; the spermatogenesis occurred during late autumn–winter (Jun–Set) and spermiogenesis was completed in spring–summer (Oct–Mar) (Table 6). The regression of the seminiferous epithelium occurred only for a short period in autumn (May). In other words, the timing of seasonal spermatogenesis was interleaved with quick episodes of testicular total

regression. This phase is commonly seen in snakes from temperate areas. Despite this fact, the neotropical snakes *Crotalus durissus* (Barros *et al*. 2012) and *Bothrops erythromelas* (Barros *et al*. 2014*b*) also exhibit a phase of testicular regression. The main cause for testicular regression in temperate species is the temperature, while in tropical species is the rainfall (Krohmer and Lutterschmidt 2011). However, in subtropical environments of south Brazil, the seasonal changes in temperature resemble temperate regions.

Therefore, the male reproductive cycle of *P. patagoniensis* may be classified according to Mathies (2011): at the individual level, males exhibit a discontinuous cyclical reproduction, with reproductively quiescence of gonads in late autumn. According to this classification, at the population level, the reproductive cycle is defined by the synchrony of reproduction observed within the male individuals. In this case, the cycles of individuals do not progress in close synchrony, but tend to be more coincident at a particular time of year (spring–summer), identifiable as a peak period of reproduction. Because of this, at the population level, we classify the reproductive cycle of *P. patagoniensis* as seasonal semisynchronous, with most of the individuals showing a reproductive peak in spring–summer. The reproductive cycle is complexly influenced by intrinsic and environmental factors. In this sense, cold winter temperatures are considered the main restriction on the duration of the reproductive season, because only in summer the temperature and insolation are high enough to allow the embryonic development (Gregory 2009).

The ductus deferens demonstrated no seasonal variation in diameter and presented spermatozoa in the lumen throughout the year. Hence, the ductus deferens is probably playing a role of long–term sperm storage, which points out to the fact that males may be capable of copulating at any time (Almeida–Santos and Salomão 1997). In this case, mating may occur independently of spermatogenesis because the male can adjust to the female reproductive cycle, being able to provide viable spermatozoa during early spring matings. The sperm storage is a synapomorphy of squamata that contributed to their successful invasion of the terrestrial environment (Gribbins, Happ and Sever 2005).

In *P. patagoniensis*, a peak in the GSI occurred during spring; nevertheless, TV appeared to be almost constant from spring to autumn, being quiescent only in winter. Meanwhile, the seminiferous tubules showed an increase in the tubule diameter and epithelial height during spring–summer. These events coincide with the time of highest activity of the testes in the spermiogenesis stage. Thus, separately, neither GSI nor testes volume should be considered a reliable indicator of spermatogenesis in *P. patagoniensis*. The histological analysis is crucial to determine the reproductive cycle (Mathies 2011).

The Leydig cells, which bordered the basal lamina of the germinal epithelium, showed an increase of the nuclear diameter in spring, typical of the mating season (Rojas *et al*. 2013). Hypertrophy in the Leydig cells nuclear diameter is associated to its endocrine activity peak of testosterone synthesis, coinciding with the spermatogenesis (Volsøe 1944). The increase of steroidogenic activity is closely associated with the transformation of peritubular cells from fibroblast to myoid–like appearance. This phenomenon suggests the involvement of Leydig cells in the sperm transport, aiding the contraction of seminiferous tubules (Kumar, Roy and Umesh 2011).

The SSK cycle follows a temporal strategy (Aldridge *et al*. 2011), in which synthesis and secretory phases are separated into non–reproductive and reproductive seasons, respectively. The increase of the tubular diameter of SSK and epithelium height during winter occurred due to the height of the secretory cells that were full of secretory granules in those seasons (Sever *et al*. 2007). Probably, the activity peak in the winter prepares the SSK for its immediate use in the mating season (spring–summer) (Aldridge *et al*. 2011; Rojas *et al*. 2013). The presence of secretory granules in the SSK tubule lumen in spring occurred associated with the mating season. After mating in spring–summer, the SSK of the *P. patagoniensis* regressed.

The smaller activity period of the spermatogenic epithelium (winter) occurred simultaneously to the period of the most activity of the SSK epithelium. The SSK secretory cycle and its relationship with the testicular cycle indicate that the development of the SSK represents a substantial energetic cost that may be equal to or greater than the cost of development of the testes (Aldridge *et al.* 2011). So we assume that SSK is also an energetically expensive structure of vital importance to reproductive biology of the *P. patagoniensis.*

Size–fecundity relationships and reproductive investment may vary even among closely related species (Shine 1988). In the case of *P. patagoniensis*, body mass had a positive relationship with TV, but not with testicular mass. In addition, the male SVL and reproductive effort (GSI) were positive correlated. The testes size is commonly hypertrophied in species with sperm competition and/or in populations with high prey availability (Møller and Briskie 1995). In this context, ecologists have classically considered that the reproductive investment is extremely different between the sexes. Even though, reproduction is energetically expensive for both males and females (Olsson, Madsen and Shine 1997). Despite this, the energy costs of sperm production are just a small component of reproduction when compared with the energy costs of reproductive behaviors (Winne and Hopkins 2006).

The *P. patagoniensis* is a widely distributed species, although information on its reproductive cycle is not available for males from other populations. But, taking account the

female reproductive cycle (Fowler *et al*. 1998; Pontes 2007; López and Giraudo 2008; Rojas, *et al*. 2015), all the populations analyzed show seasonal reproduction and some variation is recognized just in the time of reproductive events, like vitellogenesis, ovulation, oviposition and hatchling of newborns. Inter-populational variation in reproductive traits is often attributed to different climatic conditions (Pizzatto and Marques 2006; Mathies *et al.* 2010). While, the absence of variability in reproductive patterns among populations that live under different climatic conditions, like observed to *P. patagoniensis*, may be attributed to phylogenetic conservatism (James and Shine 1988). *P. patagoniensis* females from south Brazil show seasonal reproductive cycle with ovulation in spring and oviposition in summer (Pontes 2007; López and Giraudo 2008), but clearly occurs some asynchrony with the male cycle. Because of thi, the ability to sperm storage in the male ductus deferens and in the female oviduct (Rojas *et al*. 2015) evolved to allow the reproductive success of *P. patagoniensis.*

In conclusion, our results indicate that *P. patagoniensis* males have a seasonal pattern of reproduction in subtropical Brazil. The use of morpho–anatomical analysis allowed to infer a reproductive peak, but we presented evidence to support the argument that the histology was essential to reveal the precise time of some reproductive events, like sperm storage. After all, conclusions about reproductive patterns of squamate reptiles based just on morpho–anatomical methods are not very reliable. In order to analyze completely the male reproductive strategies of *P. patagoniensis*, more studies using electron microscopy and histochemistry techniques are required, as well as laboratory experiments. Therefore, we expect that this study increases the knowledge about reproduction of neotropical squamate reptiles and stimulates further research.

REFERENCES

Aldridge, R. D.; Goldberg, S. R.; Wisniewski, S. S.; Bufalino, A. P. and Dillman. C. B. 2009. The reproductive cycle and estrus in the colubrid snakes of temperate North America. *Contemporary Herpetology* 4:1–31.

Aldridge R. D.; Jellen B. C.; Siegel D. S. and Wisniewski S. S. 2011. The sexual segment of the kidney. In: *Reproductive Biology and Phylogeny of Snakes*: 477–509. Aldridge R. D.; Sever D. M. (Eds.). Enfield: Science Publishers.

Almeida–Santos, S. M. and Salomão, M. G. 1997. Long–Term Sperm Storage in the Female Neotropical Rattlesnake *Crotalus durissus*. *Japanase Journal of Herpetology* 17(2):46–52.

Almeida–Santos, S. M. and Salomão, M. G. 2002. Reproduction in neotropical pitvipers, with emphasis on species of the genus *Bothrops*. In: *Biology of the Vipers*: 445–462. Schuett, G.; Hoggren, M.; Douglas, M. E. and Greene, H. W. (Eds.). Carmel, Indiana: Eagle Mountain.

Almeida–Santos, S. M.; Laporta–Ferreira, I. L.; Antoniazzi, M. M. and Jared C. 2004. Sperm storage in males of the snake *Crotalus durissus terrificus* (Crotalinae: Viperidae) in southeastern Brazil. *Comparative Biochemistry and Physiology Part A* 139(2):169–174.

Almeida–Santos, S. M.; Braz, H. B.; Santos, L. C.; Sueiro, L. R.; Barros, V. A.; Rojas, C. A. and Kasperoviczus, K. N. 2014. Biologia reprodutiva de serpentes: recomendações para a coleta e análise de dados. *Herpetologia Brasileira* 3(1):14–24.

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

Barros, V. A.; Sueiro, L. R. and Almeida–Santos, S. M. 2012. Reproductive biology of the neotropical rattlesnake *Crotalus durissus* from northeastern Brazil: A test of phylogenetic conservatism of reproductive patterns. *Herpetological Journal* 22:97–104.

Barros, V. A.; Rojas, C. A. and Almeida–Santos, S. M. 2014*a*. Is rainfall seasonality important for reproductive strategies in viviparous Neotropical pit vipers? A case study with *Bothrops leucurus* from the Brazilian Atlantic Forest. *Herpetological Journal* 24:69–77.

Barros, V. A.; Rojas, C. A. and Almeida–Santos, S. M. 2014*b*. Reproductive Biology of *Bothrops erythromelas* from the Brazilian Caatinga. *Advances* in Zoology 2014:1–11.

Bishop, J. E. 1959. A histological and histochemical study of the kidney tubule of the common garter snake, *Thamnophis sirtalis*, with special reference to the sexual segment in the male. *Journal of Morphology* 104(2):307–357.

Braz, H. B.; Kasperoviczus, K. N. and Almeida–Santos, S. M. 2014. Reproductive ecology and diet of the fossorial snake *Phalotris lativittatus* in the Brazilian Cerrado. *Herpetological Journal* 24:49–57.

Brown, G. P. and Shine, R. 2006. Why Do Most Tropical Animals Reproduce Seasonally? Testing Hypotheses on an Australian Snake. *Ecology Society of America* 87(1):133–143.

Clesson, D.; Bautista, A.; Baleckaitis, D. D. and Krohmer R. W. 2002. Reproductive biology of male eastern garter snakes (*Thamnophis sirtalis sirtalis*) from a denning populations in Central Wisconsin. *American Midland Naturalist* 147:376–386.

Fowler, I. R. and Salomão, M. G. 1994. A study of sexual dimorphism in six species from the colubrid snake genus *Philodryas*. *The Snake* 26:117–122.

Fowler, I. R.; Salomão, M. G. and Jordão, R. S. 1998. A description of the female reproductive cycle in four species from the neotropical colubrid snake *Philodryas* (Colubridae. Xenodontinae). *The Snake* 28:71–78.

Goldberg S. R. and Parker W. S. 1975. Seasonal testicular histology of the colubrid snakes, *Masticophis taeniatus* and *Pituophis melanoleucus*. *Herpetologica* 31:317–322.

Gregory, P. T. 2009. Northern Lights and Seasonal Sex: The Reproductive Ecology of Cool–Climate Snakes. *Herpetologica* 65(1):1–13.

Granados-González, G.; Rheubert, J. L.; Villagrán-SantaCruz, M.; González-Herrera, M. E.; Dávila-Cedillo, J. V.; Gribbins, K. M. and Hernández-Gallegos, O. 2015 Male reproductive cycle in *Aspidoscelis costata costata* (Squamata:Teiidae) from Tonatico, Estado de México, México. *Acta Zoologica* 96 (1):108–116.

Gribbins, K. M.; Happ, C. S. and Sever, D. M. 2005. Ultrastructure of the reproductive system of the black swamp snake (*Seminatrix pygaea*). V. The temporal germ cell development strategy of the testis. *Acta Zoologica* 86:223–230.

Gribbins, K. M. 2011. Reptilian spermatogenesis. A histological and ultrastructural perspective. *Spermatogenesis* 1(3): 250–269.

James, C. and Shine, R. 1988. Life history strategies of Australian lizards: a comparison between the tropics and the temperate zone. *Oecologia* 75:307–316.

Krohmer, R. W.; Martinez, D. and Mason, R. T. 2004. Development of the renal sexual segment in inmature snakes: Effect of sex steroid hormones. *Comparative Biochemistry and Physiology Part A* 139:55–64.

Krohmer, R. W. and Lutterschmidt, D. I. 2011. Environmental and Neuroendocrine Control in Snakes. In: *Reproductive Biology and Phylogeny of Snakes*: 289–346. Aldridge R. D. and Sever D. M. (Eds.) Enfield: Science Publishers.

Kumar, S.; Roy, B. and Umesh, R. 2011. Hormonal Regulation of Testicular Functions in Reptiles. In: Norris D.; Lopez, K. (Ed.) *Hormones and Reproduction of Vertebrates–Reptiles*: 89–115. Academic Press, San Diego, California.

López, M. S. and Giraudo, A. R. 2008. Ecology of the snake *Philodryas patagonensis* (Serpentes: Colubridae) from Northeast Argentina. *Journal of Herpetology* 42(3):474–480.

Liang, G.; Liu, Q.; Yu, H. and Wang Q. 2011. Histological and immunocytochemical study of deferens ducts in the Chinese rat snake (*Zaocys dhumnades*). *Zoological Research* 32(6):663-669.

Mathies, T.; Cruz, J. A.; Lance, V. A. and Savidge, J.A. 2010. Reproductive biology of male brown tree snakes (*Boiga irregularis*) on Guam. Journal of Herpetology 44:209–221.

Mathies T. 2011. Reproductive cycles of tropical snakes. In: *Reproductive Biology and Phylogeny of Snakes*: 511–550. Aldridge R. D.; Sever D. M. (Eds.). Enfield: Science Publishers.

Møller, A. P. and Briskie, J. V. 1995. Extra–pair paternity, sperm competition and the evolution of testis size in birds. *Behavioral Ecology and Sociobiology* 36:35–365.

Nilson, G. and Andrén, C. 1982. Function of renal sex secretion and male hierarchy in the adder, *Vipera berus*, during reproduction. *Hormones and Behavior* 16(4):404–413.

Olsson, M.; Madsen, T. and Shine, R. 1997. Is sperm really so cheap? Costs of reproduction in male adders, *Vipera berus*. *Proceedings of the Royal Society of London Biological Sciences* 264:455–459.

Peters, J. A. and Orejas–Miranda, B. 1970. Catalogue of the neotropical Squamata. Part I – Snakes. *United States National Museum Bulletin* 297:1–347.

Pizzatto, L. and Marques, O. A. V. 2006. Interpopulational variation in reproductive cycles and activity of the water snake *Liophis miliaris* (Colubridae) in Brazil. *Herpetological Journal* 16:353–362.

Pleguezuelos, J. M. and Feriche, M. 1999. Reproductive ecology of the horseshoe whip snake (*Coluber hippocrepis)* in the Southeast of the Iberian Peninsula. *Journal of Herpetology* 33(2):202–207.

Pontes, G. M. F. 2007. História natural de *Philodryas patagoniensis* (Serpentes: Colubridae) no litoral do Rio Grande do Sul, Brasil. PhD Thesis. Pontífica Universidade Católica, Porto Alegre, Rio Grande do Sul, Brazil,

Resende, F. C. and Nascimento, L. B. 2015. The female reproductive cycle of the neotropical snake *Atractus pantostictus* (Fernandes and Puorto, 1993) from South–eastern Brazil. *Anatomia Histologia e Embryologia* 44:225–235.

Rojas, C. A.; Barros, V. A. and Almeida–Santos, S. M. 2013. The Reproductive Cycle of the Male Sleep Snake *Sibynomorphus mikanii* (Schlegel, 1837) in Southeastern Brazil. *Journal of Morphology* 274(2):215–228.

Rojas, C. A.; Barros, V. A. and Almeida–Santos, S. M. 2015. Sperm storage and morphofunctional bases of the female reproductive tract of the snake *Philodryas patagoniensis* from southeastern Brazil. *Zoomorphology* 134:1–10.

Saint Girons, H. 1982. Reproductive cycles of male snakes and their relationships with climate and female reproductive cycles. *Herpetologica* 38:5–16.

Seigel, R. A. and Ford, N. B. 1987. Reproductive ecology. In: *Snakes: Ecology and Evolutionary Biology*: 210–252. Seigel, R. A.; Collins, J. T.; Novak, S. S. (Eds.), Macmillan Publishing, New York.

Sever, D. M. 2004. Ultrastucture of the reproductive system of the black swamp snake (*Seminatrix pygaea*). IV. Occurrence of an ampulla ductus deferentis. *Journal of Morphology* 262:714–730.

Sever, D. M. and Hamlett, W. C. 2002. Female sperm storage in reptiles. *Journal of Experimental Zoology* 292:187–199.

Sever, D. M.; Siegel, S. D.; Bagwill, A.; Eckstut, E. M.; Alexander L.; Camus, A. and Morgan, C. 2007. Renal sexual segment of the cottonmouth snake *Agkistrodon piscivorous* (Reptilia, Squamata, Viperidae). *Journal of Morphology* 269:640–653.

Shine, R. 1977. Reproduction in Australian elapid snakes. II. Testicular cycles and mating seasons. *Australian Journal of Zoology* 25:647–653.

Shine, R. 1988. Constraints on Reproductive Investment: A Comparison between Aquatic and Terrestrial Snakes. *Evolution* 42(1):17–27.

Shine, R.; Olsson, M. M. and Mason, R. T. 2000. Chastity belts in gartersnakes: the functional significance of mating plugs. *Biological Journal of the Linnean Society* 70:377–390.

Statsoft, Inc. 2011. Statistica for Windows (data analysis software system), version 10.

Systat, Inc. 2011. Sigmaplot for Windows, version 12.0. Systat Software.

Vitt, L. J. and Caldwell, J. P. 2014. Reproduction and Life Histories. In: *Herpetology: An Introductory Biology of Amphibians and Reptiles*, 4nd edn. Academic Press: San Diego.

Volsøe H. 1944. Structure and seasonal variation of the male reproductive organs of *Vipera berus* (L.). *Spolia Zoologica Musei Hauniensis* 5:1–157

Winne, C. T. and Hopkins, W. A. 2006. Influence of sex and reproductive condition on terrestrial and aquatic locomotor performance in the semi–aquatic snake *Seminatrix pygaea. Functional Ecology* 20:1054–1061.

Appendix 1. Voucher specimens of males *P. patagoniensis* analyzed in this study housed in the herpetological collections of the Federal University of Santa Maria (ZUFSM) and Pontifical Catholic University of Rio Grande do Sul (MCP-PUCRS)

Macroscopic and anatomical analysis (n=85): ZUFSM0352, 0353, 0356, 0391, 0482, 0543, 0640, 0649, 0689, 0696, 0794, 0835, 0842, 0968, 0978, 0990, 1117, 1303, 1419, 1430, 1435, 1437, 1455, 1601, 1675, 1684, 1703, 1725, 1748, 1853, 2229, 2347, 2427, 2430, 2470, 2566, 2633, 2737, 2743, 2746, 2766, 2913, 3004, and 3041; MCP02503, 05499, 05753, 05759, 06474, 10992, 11043, 12525, 14263, 14277, 14336, 14482, 14774, 14878, 14897, 15679, 15680, 15681, 15682, 15847, 16722, 16946, 16947, 16948, 16949, 16951, 16961, 16962, 16963, 16964, 16969, 16970, 16971, 17018, 17859, 17955, 17966, 17988, 17989, 18065, and 18368. Histology (n=61): ZUFSM0353, 0356, 0482, 0640, 0649, 0689, 0696, 0794, 0835, 0842, 0968, 0978, 0990, 1117, 1419, 1430, 1435, 1437, 1455, 1601, 1675, 1684, 1725, 1748, 1853, 2229, 2347, 2430, 2470, 2566, 2743, 2746, 2766, and 2913; MCP14277, 14482, 14878, 14897, 15679, 15681, 15682, 15847, 16722, 16946, 16947, 16948, 16949, 16951, 16961, 16962, 16963, 16964, 16969, 16970, 16971, 17018, 17859, 17955, 17966, 17988, and 17989.

specimens under their care; We also thank the editor and anonymous reviewers for their helpful comments and suggestions.

TABLES AND FIGURES

Table 1: Macroscopic measurements of male snake *P. patagoniensis* in South Brazil. Post hoc analysis: significant differences (p<0.05) between seasons are indicated by letters. Each letter represents a season. Date are expressed as mean ± standard errors. SVL: snout-vent length; GSI: gonadosomatic index

Season	SVL (mm)	Testes volume (mm³)	GSI	Ductus deferens width (mm)	Kidney length (mm)	Kidney width (mm)
Summer (A)	653.58±38.30	295.46±48.97 [C]	1.32±0.28 [C]	1.62±0.20	58.80±7.04 [C, D]	5.64±1.84
Autumn (B)	644.30±39.78	274.17±47.61 [C]	0.65±0.18 [C]	1.44±0.24	62.02±2.69 [C]	6.23±1.96
Winter (C)	636.86±26.28	183.00±62.40 [A, B, D]	0.41±0.08 [A, B]	1.64±0.25	64.09±3.48 [A, B]	5.50±2.07
Spring (D)	649.46±23.22	256.49±22.87 [C]	1.88±0.45 [B, C]	1.85±0.22	63.57±5.10 [A]	5.79±1.70

Adapted from Rojas *et al.* 2013.

Table 2: Structural variation of the testes of *P. patagoniensis* between different seasons. Post hoc analysis: significant differences (p<0.05) between seasons are indicated by letters. Each letter represents a season. Date are expressed as mean ± standard errors.

Season	Seminiferous tubule diameter (μm)	Seminiferous epithelial height (μm)	Leydig cell nuclear diameter (μm)
Summer (A)	287.82±13.50 [C, D]	105.45±6.53 [C, D]	3.73±030 [D]
Autumn (B)	253.29±13.35	90.29±4.47	3.93±0.27 [D]
Winter (C)	218.29±12.32 [A]	75.14±4.21 [A]	3.82±0.23 [D]
Spring (D)	233.23±9.19 [A]	86.13±3.43 [A]	4.92±0.24 [A, B, C]

Adapted from Rojas *et al.* 2013.

Table 3: Stages of the spermatogenic cycle in *P. patagoniensis* in South Brazil

Stages	Months of occurrence
I) Complete regression	May
II) Early recrudescence: division of spermatogonia and primary spermatocytes	June-July
III) Late recrudescence: primary spermatocytes and spermatids	July - September
IV) Early spermiogenesis: spermatids in metamorphosis	October - January
V) Spermiogenesis: mature spermatoza in the lumen	November - March
VI) Early regression: decrease of the seminiferous epithelium	April - May

Adapted from Goldberg and Parker (1975).

Table 4: Structural variation of the kidney of *P. patagoniensis* between different seasons. Post hoc analysis: significant differences (p<0.05) between seasons are indicated by letters. Each letter represents a season. Date are expressed as mean ± standard errors. SSK: sexual segment of kidney.

Season	SSK tubular diameter (µm)	SSK epithelial height (µm)
Summer (A)	160.11±9.18 [C]	68.77±3.95 [C]
Autumn (B)	176.62±7.75	81.14±5.06
Winter (C)	197.78±9.75 [A, D]	92.33±7.55 [A, D]
Spring (D)	169.30±4.7 [C]	73.00±3.21 [C]

Adapted from Rojas *et al.* 2013.

Table 5: Stages of the SSK cycle in *P. patagoniensis* in South Brazil.

Stages	Months of occurrence
0) SSK not hypertrophied	March- April
1) SSK hypertrophied with a few granules	May-June
2) SSK cytoplasm full of secretory granules	July-September
3) SSK secretory granules apically in the cytoplasm	September-November
4) Maximum density of SSK secretory granules	December-February

Adapted from Krohmer *et al.* (2004).

Table 6: Phases of the annual reproductive cycle of male *P. patagoniensis* in South Brazil

	Summer	Autumn	Winter	Spring
Testes hypertrophy (volume)	▓	▓		▓
GSI increases				▓
Spermatogenesis				▓
Spermiogenesis	▓			
Seminiferous tubule hypertrophy	▓			
Leydig cell activity				▓
Kidney hypertrophy (length)	▓	▓		
SSK hypertrophy			▓	
Testicular regression		▓		
Mating	▓			▓
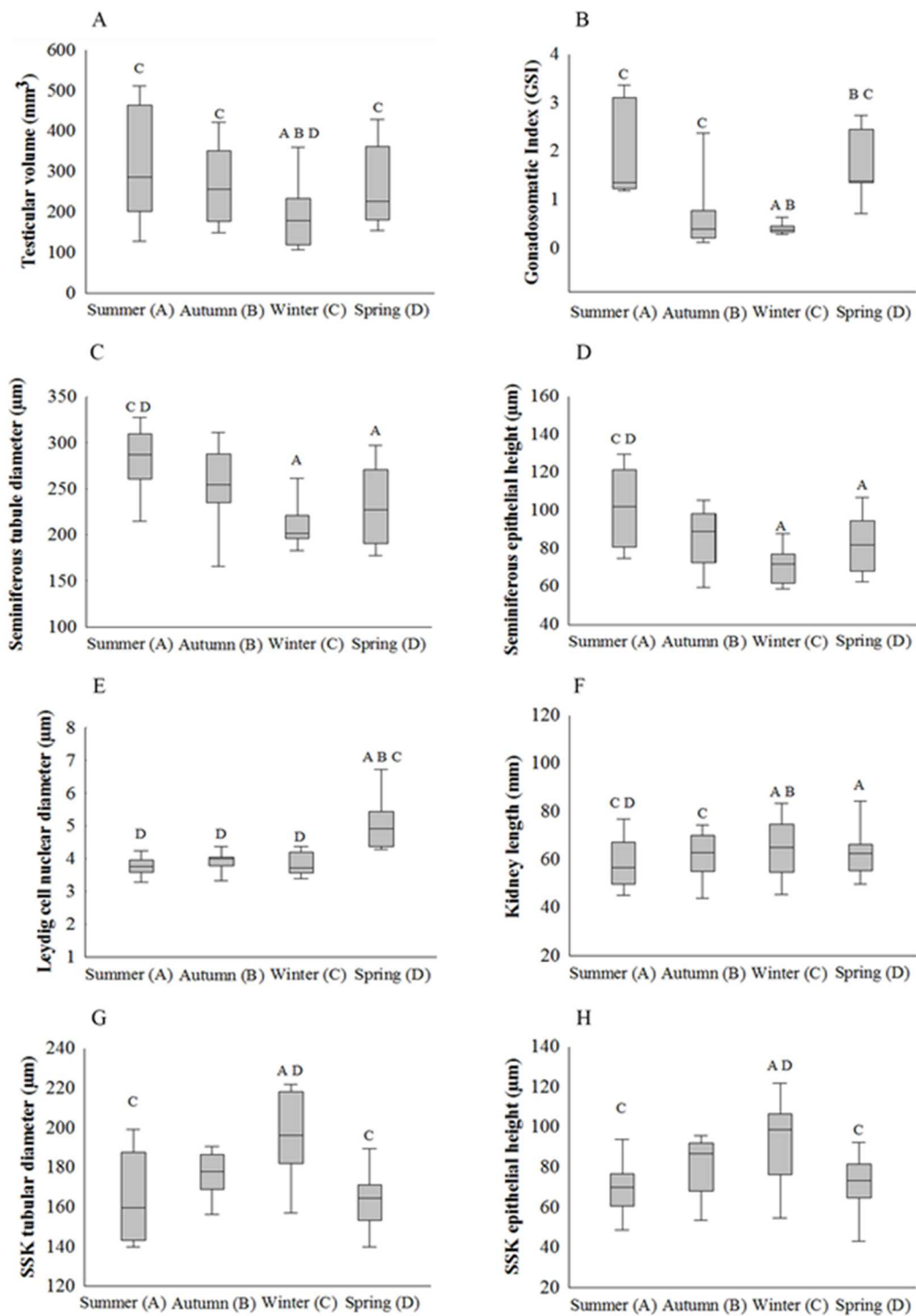
Figure 1. Seasonal variation in (A) testicular volume, (B) GSI, (C) kidney length, (D) seminiferous tubule diameter, (E) seminiferous epithelial height, (F) Leydig cell nuclear diameter, (G) SSK tubular diameter and (H) SSK epithelial height for males of *P. patagoniensis* in South Brazil. Middle line represents mean values, boxes show standard deviation and whiskers represent minimum and maximum values. Each letter express differences between the seasons.
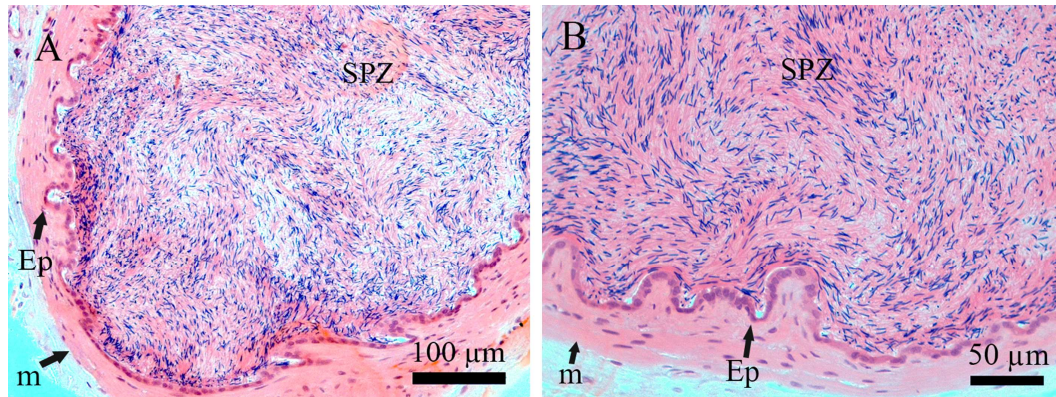
Figure 2: Transverse sections of the ampulla of the ductus deferens of *P. patagoniensis.* (A) ductus deferens lumen full spermatozoa; (B) detail of the ductus deferens epithelium. Ep: epithelium; m: muscularis; SPZ: spermatozoa;
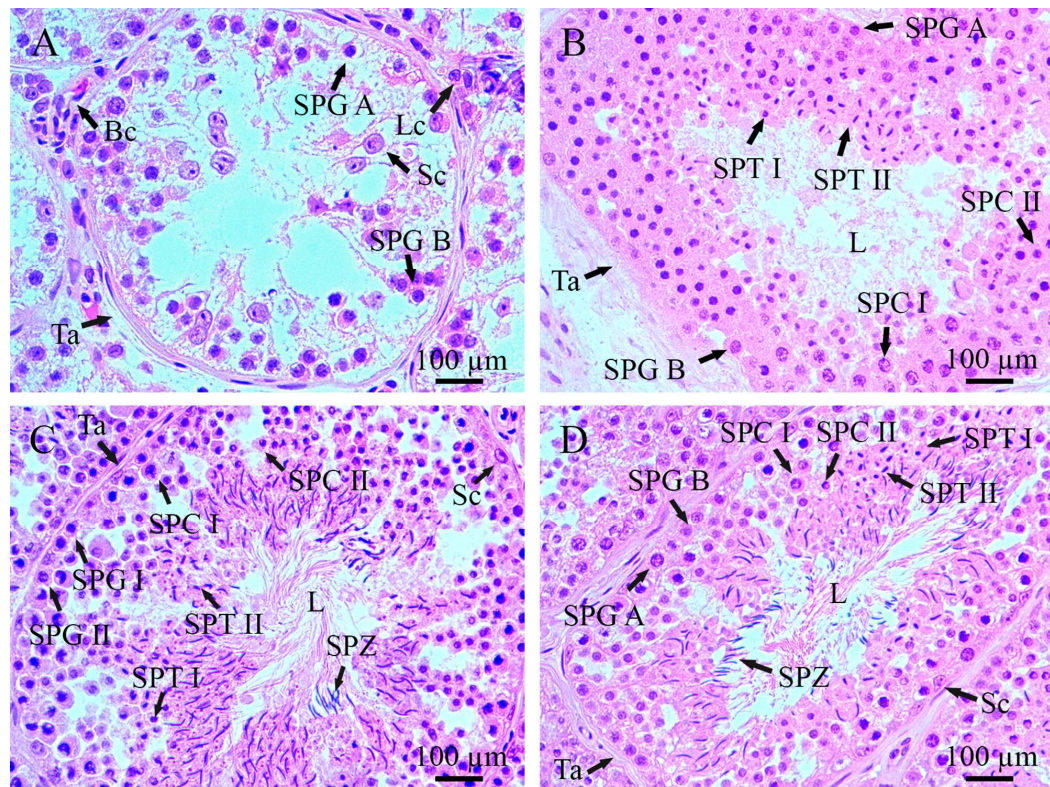
Figure 3. Transverse sections of the testes of *P. patagoniensis* from South Brazil in (A) autumn (May) seminiferous epithelium in regression; (B) in winter (July) showing different stages of cellular division in the seminiferous epithelium; (C) in spring (October) with the peak of cellular division and spermatid in metamorphosis; (D) in late summer (March), final phase of the spermiogenesis process. Ta: tunica albuginea; Bc: blood capillaries; Lc: Leydig cells; Sc: Sertoli cells; L: lumen; Sd: spermatid; SPG A: spermatogonia A; SPG B: spermatogonia B; SPC I: primary spermatocyte; SPC II: secondary spermatocyte; SPT I: spermatid I; SPT II: spermatid II; SPZ: spermatozoa;
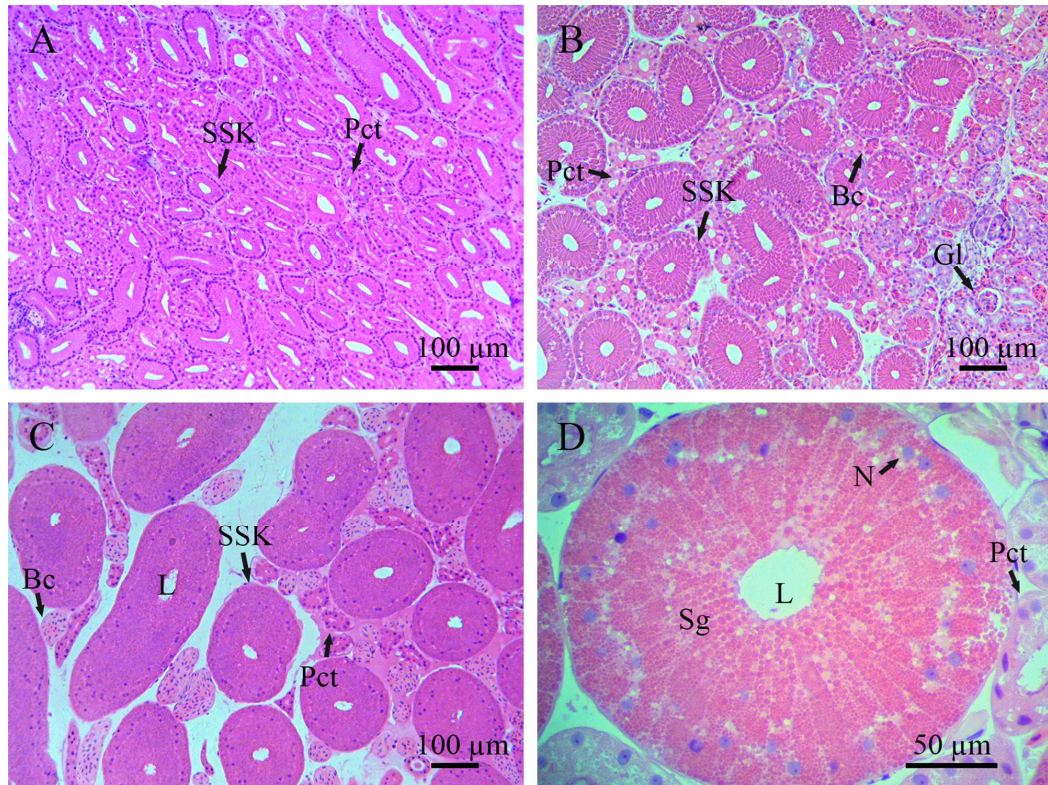
Figure 4: Histology of the kidney of the males of *P. patagoniensis* from South Brazil: (A) SSK regressed (late summer - early autumn); (B) SSK in hypertrophy with granules evident throughout the cytoplasm (winter); (C) SSK with secretory granules visible in the apical region of the cytoplasm (late winter - spring); (D) detail of SSK cells full of secretory granules maximum density of secretory granules within the cytoplasm (late spring - summer). Bc: blood capillaries; Sg: secretory granules; Gl: kidney glomerulum L: lumen; Pct: proximal convoluted tubule; SSK: sexual segment of the kidney; N: cell nucleum;

Figure 5: Relationship between (A) testicular volume and kidney length, (B) seminiferous tubule diameter vs. SSK tubule diameter and (C) and seminiferous epithelial height vs. SSK epithelial height for males of *P. patagoniensis* in South Brazil.

Figure 6: Relationship between (A) body mass and testicular volume, and (B) body size and gonadosomatic index for males of *P. patagoniensis* in South Brazil.

2 REPRODUCTIVE BIOLOGY OF THE *PHILODRYAS PATAGONIENSIS* (SNAKES: DIPSADIDAE) IN SOUTH BRAZIL: FEMALE REPRODUCTIVE CYCLE

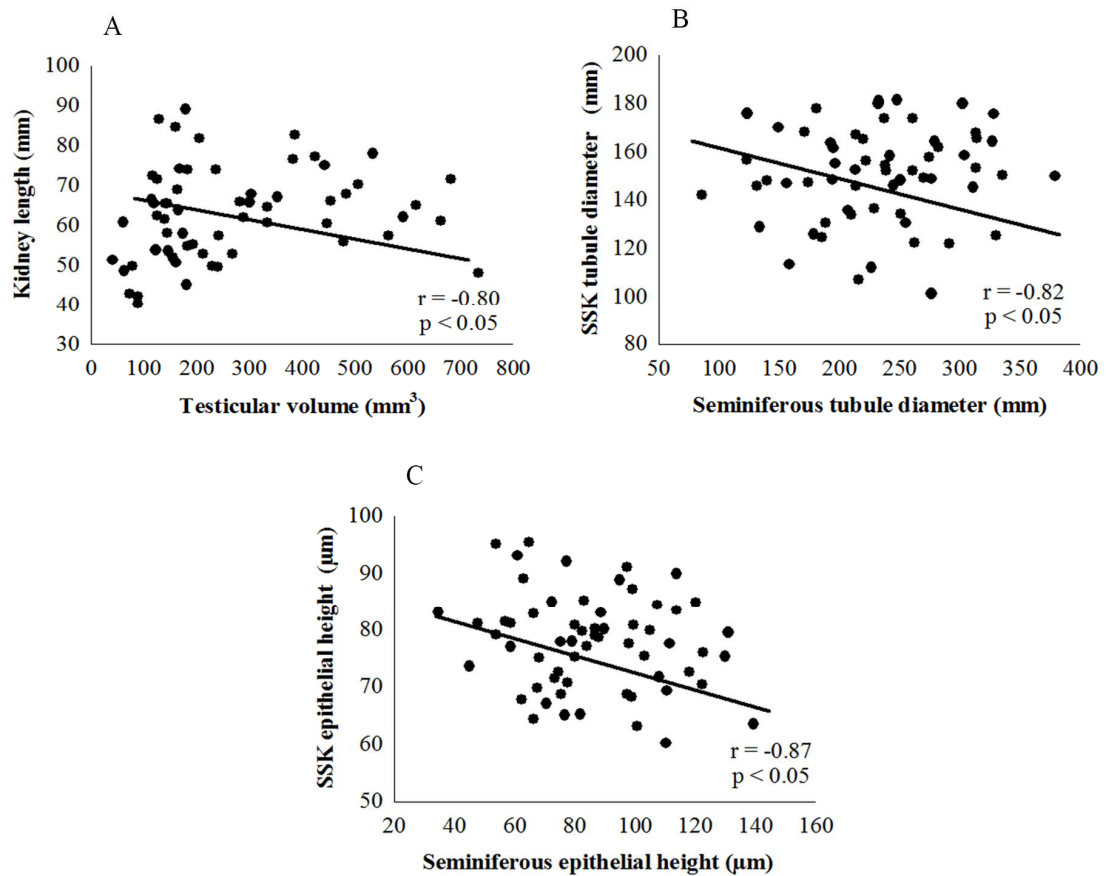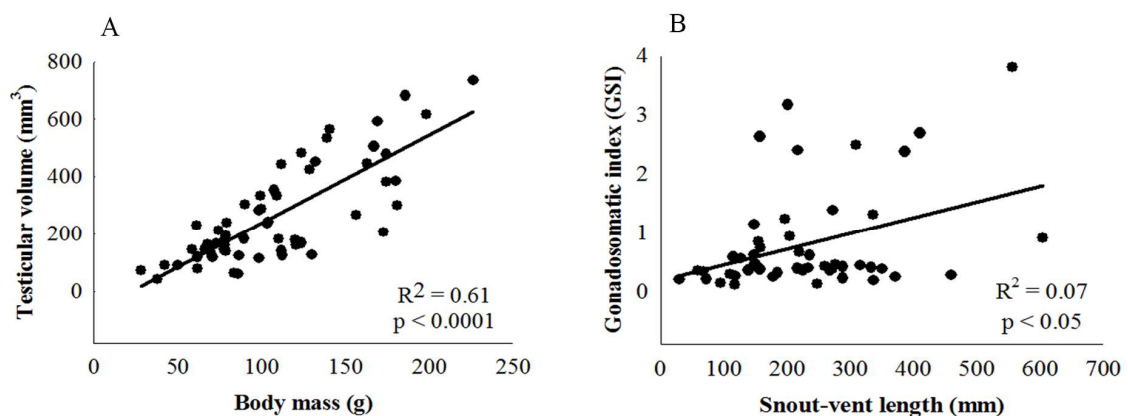## CICLO REPRODUTIVO DE *PHILODRYAS PATAGONIENSIS* (SERPENTES: DIPSADIDAE) NO SUL DO BRASIL: CICLO REPRODUTIVO DAS FÊMEAS

Luiza Loebens[1*], Claudio Augusto Rojas[2], Sonia Zanini Cechin[1], Selma Maria Almeida-Santos[2]

ABSTRACT

The *Philodryas. patagoniensis* female reproductive cycle in South Brazil is described through morpho-anatomical and histological analysis of individuals deposited in zoological collections. The *P. patagoniensis* females remain reproductively active during an extended period of the year, being vitellogenic follicles evidenced in all the seasons and ovigerous females from spring to autumn (Oct-Jun). The secondary vitellogenesis peak occurs during winter-spring (Jul-Dec), ovulation in spring (Oct-Dec), mating and fertilization in spring-summer (Oct-Feb), oviposition in spring-autumn (Oct-May), and births from late spring to autumn (Dec-Jul). The diameter of vitellogenic follicles/eggs were larger in winter-spring, the same period in which ovaries were in hypertrophy. The diameter of the shell glands also was larger in winter-spring. In spite of the reproductive peak clearly evidenced, gonads only revealed reduced activity in autumn. So, at the individual level females has a discontinuous cyclical reproduction, and considering the population level, the reproductive cycle is seasonal semisynchronous. We support the theory that *P. patagoniensis* have ability to produce multiple clutches with sperm long-term stored. Sexual body size dimorphism was evident being females significantly larger and heavier than males. *P. patagoniensis* larger females were able to produce follicles and eggs in greater amount and size. The maternal body size was related to the reproductive effort and fecundity. In conclusion, we deliberate about the proximal and distal causes that influence the *P. patagoniensis* reproductive traits and patterns.

[1] Laboratory of Herpetology, Santa Maria Federal University, Santa Maria, Rio Grande do Sul, Brazil

[2] Laboratory of Ecology and Evolution, Butantan Institute, São Paulo, São Paulo, Brazil

[*] Corresponding author: Laboratory of Herpetology, Santa Maria Federal University (UFSM), Santa Maria, 1000 Roraima Avenue, Camobi, Santa Maria, Rio Grande do Sul (RS) 97105-900, Brazil; e-mail address:loebens.luiza@gmail.com.

KEYWORDS: reproduction; vitellogenesis; sperm storage; seasonal activity; sexual dimorphism.

RESUMO

O ciclo reprodutivo das fêmeas de *Philodryas patagoniensis* no sul do Brasil é descrito através de análises morfoanatômicas e histológicas de indivíduos depositados em coleções zoológicas. As fêmeas de *P. patagoniensis* permanecem reprodutivamente ativas durante um longo período do ano, sendo evidenciados folículos vitelogênicos em todas as estações do ano e fêmeas ovígeras da primavera ao outono (Out-Jun). O pico da vitelogênese secundária ocorre no período inverno-primavera (Jul-Dez), a ovulação na primavera (Out-Dez), acasalamento e fertilização durante primavera e verão (Out-Fev), ovoposição no período primavera-outono (Out-Mai) e nascimentos do final da primavera ao outono (Dez-Jul). O diâmetro dos folículos vitelogênicos/ovos foi maior no período inverno-primavera, ao mesmo tempo em que os ovários apresentaram maior massa. O diâmetro das glândulas da casca também apresentou aumento no inverno. Apesar do pico reprodutivo ter sido claramente evidenciado, as gônadas revelaram atividade reduzida somente no outono. Então, em nível de indivíduo as fêmeas exibem um ciclo reprodutivo cíclico descontínuo, enquanto a nível populacional o ciclo reprodutivo é sazonal semi-sincrônico. *P. patagoniensis* tem capacidade para produzir múltiplas desovas com esperma estocado a longo prazo. Evidenciou-se dimorfismo sexual no tamanho do corpo, sendo as fêmeas significativamente maiores e mais pesadas do que os machos. As fêmeas maiores são capazes de produzir folículos e ovos em maior quantidade e tamanho. O tamanho corpóreo materno relaciona-se diretamente com o esforço reprodutivo e a fecundidade. Por fim, discutimos causas proximais e distais que influenciam as caraterísticas e o padrão reprodutivo de *P. patagoniensis*.

PALAVRAS-CHAVE: reprodução; vitelogênese; estocagem de esperma; atividade sazonal; dimorfismo sexual.

2.1 INTRODUCTION

The major motivation of the life-history theory is to explain the amazing differences in life-history strategies within and among the taxa in the natural world. The life-history theory attempts to describe how the natural selection and evolution compel organisms to improve their "Darwinian fitness" (Stearns *et al*. 2000). The fitness is related to the success of reproduction,

although it is not a cheap endeavor and their physiological costs may be translated into negative relationships between two life history traits known "trade-offs", such as "fecundity vs. size at maturity" and "present fecundity vs. future fecundity" (Ford and Seigel 1989). So, to understand why and how snakes use the limited energy and resources available, we should analyze many "trade-offs" involved in maximize the survival and reproduction and therefore optimize the fitness trade-offs (Fabian and Flatt 2012).

The life-history strategies developed for an organism are directly related to the adaptation of local conditions (Vitt and Caldwell 2014). In this sense, some authors now consider snakes like "model organisms" especially for studies on reproductive ecology (Shine and Bonnet 2000). Although, the snakes reproduction studies have extensively examined a small number of temperate species, while the neotropical species have received more attention just in the past two decades (Almeida-Santos *et al*. 2014). Whereas, reproduction information about a large number of species is required to understanding the evolution of reproductive traits and patterns (Barros *et al*. 2012). Moreover, natural history information is important to the definition of conservation measures (Shine and Bonnet 2009).

In snakes, many aspects of the female reproductive cycle occur independently of the male cycle (Taylor and DeNardo 2011). In general, the females cycles tend to be less variable than males, being the vitellogenesis, ovulation, and oviposition the most important events used to classify the reproductive cycle (Aldridge *et al*. 2009). Further, these events may diverge drastically depending on: phylogeny, temperature, photoperiod, reproductive season duration, food availability, body condition, and quality of fat reserves (Naulleau and Bonnet 1996). Summarizing, the plasticity in life-history traits is influenced by environmental factors as well as genetic causes (Seigel and Ford 2001), and the squamates can exhibit variation in reproductive tactics even in the intraspecific level (Barros *et al*. 2012).

The annual activity pattern is an ecological aspect associated to the reproductive biology, once it has great implications for the reproduction, and so for the species persistence (Torello-Viera *et al*. 2012). When and how long to play the reproductive endeavors (e.g. partner searching, mating and, egg-laying) during the year are another "trade-offs" to be evaluated for an organism. Furthermore, we must consider that several another abiotic and biotic factors may constrain the reproduction, and so the activity patterns (Shine 1977; Marques *et al*. 2000). In this context, Gibbons and Sernlitsch (1987) suggest two kinds of seasonal activity patterns for snakes: unimodal, in which a single peak occurs between the late spring and late summer; and bimodal in which two peaks of activity occur, one in spring and another in summer. However,

activity patterns may vary among populations and even among individuals, and not always this classification in close categories should be the more satisfactory.

An important event used to classify the reproductive cycles is the seasonal association of mating and gonadal activity (Crews 1984). In brief definition, species showing associated reproductive pattern mate while gonadal activity is elevated, whereas species with dissociated reproduction mate when gonads are quiescent. In snakes with dissociated cycle the timing of mating and fertilization are asynchronous, being necessary perform a sperm storage mechanism that allows the fertilization to be delayed (Schuett 1992).

Female sperm storage structures have evolved independently for different times in squamate (Sever and Hamlett 2002), and many explanations are proposed for this: dissociated reproductive events; scarcity of sexual partners; allow the female to lay more than one egg-clutch in the same reproductive season; less predation risk linked to the reproductive endeavors; and sexual selection through spem competiton (Tryon 1984; Schuett 1992; Birkhead and Moller 1993; Almeida-Santos and Salomão 1997).

P. patagoniensis (Girard 1858) is a diurnal and terrestrial species (Hartmann and Marques 2005) widely distributed in savannahs and grasslands of Brazil, Bolivia, Paraguay, Argentina and Uruguay (Peters and Orejas-Miranda 1970). It has a generalist diet feeding on fishes, frogs, lizards, snakes, birds, mammals (Hartmann and Marques 2005) and even their own species (Pontes *et al*. 2003). It is an oviparous snake that reproduces seasonally. Studies on female reproductive cycle have been done on Brazilian (Fowler *et al*. 1998; Pontes 2007; Rojas *et al*. 2015) and Uruguayan (López and Giraudo 2008) populations, although just Rojas *et al.* (2015) explore the histology of the female oviduct.

So, the present study describe the reproductive biology of the female snake *P. patagoniensis* in South Brazil through morphological and histological analysis, testing the following hypothesis: (1) the species should have a seasonal pattern of reproduction in the subtropical region; (2) *P. patagoniensis* females should be able to produce multiple clutches in the same reproductive season; (3) the oviduct of *P. patagoniensis* must to have sperm storage adaptations; (4) the seasonal activity pattern of the species ought to be strongly determined by the reproductive cycle (5) the female-biased sexual dimorphism in body size may be related to fecundity selection in this species.

2.2 METHOD

Data collection

We analyzed individuals of *P. patagoniensis* from the South region of Brazil (Rio Grande do Sul, Paraná and Santa Catarina States) available in the herpetological collections of the of the Federal University of Santa Maria (ZUFSM) and Pontifical Catholic University of Rio Grande do Sul (MCP-PUCRS).

The climate in the South region of Brazil is represented by Köppen climate classification as humid subtropical (Cfa and Cfb). The rains are well distributed throughout the seasons (mean annual precipitation 1.000 a 2.000 mm), so it is not possible identify a dry season. Though, the region shows a well-defined temperature seasonality, with temperatures achieving 0°C in the winter and almost 40°C in summer (Alvares *et al*. 2013).

We considered the monthly abundance of males, females and juveniles available in the collections to explore the seasonal activity pattern. For this, we analyzed 783 specimens, considering the data: sex, month of capture, and sexual maturity. We considered mature the males larger than 440 mm of snout-vent length and 690 mm of total length (Loebens, *et al*. in press). The females sexual maturity, was determined using the presence of spermatozoa in the vagina or oviduct (post-copulating) and/or the presence of mature follicles in ovary or eggs in oviduct as the main criterions, and the aspect of the oviduct with folds or loose (post-spawning) was used as a secondary criterion (Shine 1977).

Reproductive events were described according to austral seasons: summer (late December-late March), autumn (late March-late June), winter (late June-late September), and spring (late September-late December). The reproductive cycle of mature females was analyzed considering, morpho-anatomical and histological changes ovary and oviduct (infundibulum anterior and posterior, uterus, utero-vaginal junction, and vagina) (Rojas *et al*. 2015).

We analyzed 112 *P. patagoniensis* females to investigate the morpho-anatomical changes in the reproductive organs. For each individual, we obtained information on month of death, snout-vent length (SVL) and body mass (BM). We exanimated the morpho-anatomical variables: number of vitellogenic follicles and/or eggs; diameter of the largest follicle and/or oviductal eggs; and the overall condition of the uterus (opaque or translucent and smooth or relaxed). To investigate size-fecundity relationships and reproductive investment we measured the ovary mass (follicles) and calculated the Gonadosomatic Index (GSI) by the formula gonad mass/body mass x 100 (Clesson *et al*. 2002).

Last, to investigate sexual dimorphism we measured for 57 males and 46 females (mature individuals) the following external morphological traits: snout-vent length (SVL), tail length (TL) and body mass (BM). We calculated the degree of sexual dimorphism (SSD) in SVL using the formula: SSD = 1- (mean female SVL/mean male SVL) (Shine 1994).

Histology

We dissected 55 *P. patagoniensis* females to obtain histological samples. For each individual, we obtained information on month of death, snout-vent length (SVL) and body mass (BM). We obtain histological samples of the: infundibulum (anterior and posterior), uterus, utero-vaginal junction, and vagina (Rojas *et al*. 2015). As a standard procedure, only right side organs were used in this study, preserving the contralateral. The tissue samples were processed for light microscopy by histological resin (methacrylate – Leica Historesin) method. Sections taken at 2µm thick (Leica RM2245 microtome). Slides were analyzed using a ZEISS Axio Scope.A1 microscope with camera Axiocam MRc 5. Morphometric measurements and pictures were taken via ZEISS microscope software ZEN lite (Carl Zeiss 2014).

The oviduct was analyzed for the presence of spermatozoa in the different regions of the oviduct and the existence of sperm storage glands/tubules. The microscopic measurements taken: shell glands (uterus) diameter (Siegel and Sever 2008). We obtained 10 measures of this microscopic variable to each individual, considering only glands presenting circular forms in the transverse section.

Statistical analysis

The reproductive cycle was determined based on the seasonal variation in vitellogenic follicles/eggs diameter, GSI and shell glands diameter. These variables were investigated by analysis of variance (ANOVA) and significant results examined by Post-hoc tests (Tukey).

Seasonal activity was inferred through the monthly abundance of males, females and juveniles. To verify an abundance peak of individuals, we used adherence G-test (goodness of fit) with expected equal proportions for males, females and juveniles. To investigate differences in the activity pattern between adults-juvenile and males-females we performed contingency G-tests (likelihood ratio). To evaluate activity synchrony among adults-juveniles and males-females we performed a correlation tests (Pearson's).

To investigate size-fecundity relationships we used linear regressions using only BM as the predictor variable, because SVL and BM were strongly correlated (r=0.89; p<0.0001). Therefore, we analyzed the following variables relationship: BM vs. ovary mass; BM vs. number of follicles/eggs; and BM vs. follicle/egg diameter. Correlations between body size (SVL) and the values of GSI were tested through Pearson correlation coefficient. The association between female SVL and clutch size (number of oviductal eggs) was analyzed through linear regression.

The fecundity was analyzed comparing the potential fecundity (number of secondary follicles) and the observed fecundity (number of eggs in the oviducts) through a t-test (Oliveira *et al*. 2011). We investigated the relationship follicles/eggs number vs. follicles/eggs size using correlation analysis (Pearson).

To analyze sexual dimorphism we compared SVL between the sexes using analyses of variance (ANOVA). Because TL and BM vary with body length, we used SVL as a covariate in analyses of covariance (ANCOVA) to compare these variables between the sexes (Shine 1994).

Statistical analyses were performed using Statistica version 10 (Statsoft 2011), assuming p<0.05 as the criterion for significance). All variables were tested for normality and homogeneity of variances before analysis and were log-transformed if necessary (Zar 1999). The plots were created on Sigmaplot 12 (Systat 2011).

2.3 RESULTS

Seasonal activity

We obtained information on the month of capture for 280 males, 284 females, and 219 juveniles. Individuals were found during every month of the year, with a strong peak in summer to juveniles (G=68.14; p<0.0001) and in spring for males (G=73.11; p<0.0001) and females (G=152.95; p<0.0001) (Fig. 1). Males and females presented synchrony in activity during the year (r=0.83; p<0.005). There was a significant difference in the seasonal activity of adults and juveniles (G=55.89; p<0.0001). There was no synchronization between the activity of adults and juveniles (r=0.23; p>0.05).

Vitellogenic cycle

Seasonal variation in some parameters of the female reproductive cycle was identified. The diameter of vitellogenic follicles/eggs exhibited an increase during winter and spring (F=11.47; p<0.00001; Table 1; Fig. 2A). Follicles/eggs were larger during winter and spring comparing to autumn (both p<0.005) and summer (both, p<0.05). The GSI differed among the seasons (F = 6.26; p < 0.005; Table 1; Fig. 2B) and ovaries were heavier in winter and spring in comparison to autumn (both, p<0.05) and summer (both, p<0.05). Considering the microscopic data, variation in the diameter of the shell glands was seasonal (F=7.80; p<0.005; Table 1; Fig. 2C) and it was larger in winter and spring compared to summer (p<0.01 and p<0.05; Table 1). So, the shell glands in the anterior uterus of *P. patagoniensis* should be seen atrophy during summer-autumn (Fig. 3A-B) and in hypertrophy during winter-spring (Fig. 3C-D).

Follicles in reproductively active females are larger than 11.71 mm in diameter. Females with vitellogenic follicles were found in all the seasons, despite this, vitellogenesis becomes more evident by late winter to spring. Ovigerous females (n=26) were seen from spring to autumn (October, n=9; November, n=8; December, n=3; January, n=1; February, n=2; March, n=1; April, n=1; July, n=1.). Females presenting mature follicles and eggs simultaneously were found from spring to autumn (January, n=1; February, n=2; March, n=1; April, n=2; October, n=2; November, n=5; December, n=1.). Egg-clutches (n=13) were recorded from spring to early autumn (January, n=2; February, n=1; March, n=1; May, n=1; October, n=1; November, n=5; December, n=2.). Neonates were recorded from late spring (December) to early winter (July).

Vitellogenesis is extended through the year occurring the vitellogenic peak from winter to spring (July-December) and ovulation in spring (October-December), clutches were recorded from spring to autumn (October-May) and births from late-spring to early winter (December-July). Females presenting sperm in the vagina (Fig. 4B) were seen in spring and early summer (October, n=2; November, n=1; December, n=2; January, n=3; February, n=2).

Sperm storage

In *P. paragoniensis* we could identify sperm storage in two different locations in oviduct: utero-vaginal junction and posterior infundibulum. Sperm storage occurs in the in pregnant, and primary and secondary vitellogenic females. Females presenting sperm in furrows on the utero-vaginal junction (Fig. 4A) were found mainly from winter to summer

(January, n=2; April, n=1; June, n=2; July, n=2; August, n=4; October, n=3; November, n=3; December, n=1). The furrows in utero-vaginal junction were filled with undulating parallel arrays of sperm tails. Sperm nuclei are seen oriented toward the epithelium of the sperm storage receptacle. We could also identify sperm storage tubules in the posterior infundibulum in all the seasons (January, n=1; February, n=4; March, n=1; April, n=2; May, n=5; July, n=1; August, n=2; September, n=1; October, n=1; November, n=2; December, n=1). The presence of sperm stored in the sperm storage receptacles was not identified, we just found tubules with sperm debris (Fig. 4C-D) mainly in spring and summer (January, n=1; February, n=2; June, n=1; November, n=3).

Size-fecundity relationships

BM had a significant effect (r=0.48; p<0.005) and relationship (R^2=0.21; F=15.12; p<0.0005) with the ovary mass (Fig. 5A). The mean ovary mass was 6.07±9.84 g (range 0.12-44.49). The BM had a weak but significant relationship with the number of follicles/eggs (R^2=0.07; F=5.13; p<0.05; Fig. 5B). The mean clutch size (number of eggs in the oviducts) was 12.81±0.90 (range 5-22) while the average number of secondary follicles in ovary was 18.40±1.18 (range 6-40). There was a positive correlation between female SVL and clutch size (R^2=0.43; F=18.45; p<0.0005; Fig. 5C). The diameter of follicles/eggs showed a relation with BM (R^2=0.16; F=10.76; p<0.005; Fig. 5D). The average length of was secondary follicles 16.61±8.55 mm (range 11.71-25.71) and oviductal eggs 36.02±6.51 mm (range 23.60-51.94). The overall mean of the GSI for females was 2.12±0.21 (range 0.19-9.10). There was a significant positive correlation between female SVL and reproductive effort (GSI) (r=0.36; p<0.01; Fig. 5E).

The potential fecundity (secondary follicles) was significantly higher than the observed fecundity (eggs) (t=3.54; p<0.005; Fig. 6A). The follicles/eggs number was negative related to the follicles/eggs size (r=-0.44; p<0.000; Fig. 6B).

The degree of SSD was 0.37 indicating that the mean SVL of the mature females was 36.86% greater than for the mature males. The *P. patagoniensis* females reach sexual maturity at a larger size than males. The mature females SVL ranged from 500 to 1480 mm (x=915.02), TL 147 to 410 mm (x=267.87), total length from 810 to 1447 mm (x=1156.49), and BM from 117.9 to 604 (x=262.17). The SVL of the mature males ranged from 440 to 840 mm (x=668.57), TL from 150 to 370 mm (x=279.55), total length from 690 to 1180 (x=948.15), and BM from 61.1 to 354 (x=117.53). Sexual body size dimorphism was evident in mature individuals, being

females significantly larger (F=97.72; p<0.0001) and heavier (F=8.35; p<0.005) than males (Fig. 7A). Although, males presented longer TL than females (F=10.23; p<0.005 Fig. 7B).

2.4 DISCUSSION

P. patagoniensis exhibited a prolonged period of time during which females were ovigerous and with similar size distributions of vitellogenic follicles, therefore gonads only revealed reduced activity in autumn. So, we can summarize the reproductive cycle according to Mathies (2011): at the individual level females showed a discontinuous cyclical reproduction; and considering the population level, the reproductive cycle is seasonal semisynchronous with the most of the individuals showing a reproduction peak in spring-summer. Briefly, the *P. patagoniensis* reproductive cycle in South Brazil is characterized by the peak of secondary vitellogenesis occurring from winter to spring (July-December), ovulation in spring (October-December), mating and fertilization in spring and early summer (October-February), oviposition from spring to autumn (October-May), and births from late spring to early winter (December- July) (Table 2). Females presenting sperm in the vagina were considered as data of recent mating (Sever *et al*. 2000), cause direct evidence of timing of mating (e.g., observations in the wild) is not available for *P. patagoniensis.* In the southeastern Brazil *P. patagoniensis* also presented a seasonal pattern of reproduction, with secondary vitellogenesis occurring from winter to spring, ovulation in spring, oviposition, in spring-summer, and hatching of newborns in summer (Fowler *et al*. 1998; Rojas 2013).

The extended period of recruitment probably is observed due the occurrence of two birth seasons happening successively. The first birth season occurs from late spring to summer (December-March), while the second season occurs from autumn to early winter (April-July). This considering the ability of multiple clutches production with sperm long-term stored. During the non-reproductive season, the females exhibited secondary follicles in early stages of development and uterus with glandular atrophy. The values of GSI, follicle/eggs diameter and shell (uteine) glands diameter were increased during winter-spring, coinciding with the reproductive season. In females which had secondary follicles in late development, the glands were hypertrophied and full of secretory granules. A recent histochemical study of the *P. pataginiensis* oviduct revealed hypetrophy of the uterine glands and increasing production of secretory granules in the uterine epithelium during the secondary vitellogenesis (Rojas *et al*. 2015). The uterine glands have an important role in the development of eggs in oviparous

snakes once are involved in providing the pseudokeratin component of the eggs shell membrane (Hoffmann 1970; Perkins and Palmer 1996).

The ability to store sperm for more than one season might be related to sperm storage structures identified in caudal (utero-vaginal junction) and cranial location (posterior infundibulum) of the oviduct of *P. patagoniensis*. Females showed sperm groups stored in the furrows of the utero-vaginal junction in all the seasons, but in high frequency from winter to summer (July-March). Although, the occurrence of sperm storage in the posterior infundibulum was deduced merely by the occurrence of sperm storage receptacles showing sperm debris from spring to summer (October-March). Similar results were found to *P. patagoniensis* from South Brazil by Rojas (2013) analyzing sperm storage patterns in Xenodontinae. Although, in the *P. patagoniensis* from southeastern Brazil, sperm storage was clearly evidenced in the posterior infundibulum and utero-vaginal junction (Rojas *et al*. 2015). Therefore, we conclude that long-term sperm storage exists in deep furrows (crypts) of the utero-vaginal junction, while short-term sperm storage occurs in the infundibulum receptacles. In species with infundibular storage, first sperm remains stored in the utero-vaginal junction for some period of time until cranial migration to infundibulum receptacles just prior fertilization (Fox 1956; Hoffman and Wimsatt 1972; Perkins and Palmer 1996; Almeida-Santos and Salomão 1997). Consequently, the storage of sperm in infundibulum receptacles occurs until vitellogenic follicles achieve the ovulatory condition and arrive the infundibular lumen. Sperm storage structures in the utero-vaginal junction have been described as glands highly unspecialized and not differentiated from the oviduct epithelium (Sever and Ryan 1999) that act physical protecting sperm from getting swept caudally in the oviduct by the eggs (Aldridge 1992). The long-term sperm storage in the utero-vaginal junction of *P. patagoniensis* is not related to the uterine muscular twisting like neotropical pitvipers (Almeida-Santos and Salomão, 2002). The fertilization success of sperm stored to the time of ovulation is not clearly assessed, although sperm can reside in the utero-vaginal junction for as little as a few weeks, to a long as over hibernation (Siegel *et al*. 2011). In *T. sirtalis* uterus, the sperm could survive at least one month (Rahn, 1940). The presence of sperm stored in the utero-vaginal junction of pregnant and recently oviposited females suggests that *P. patagoniensis* does not eliminate the sperm after ovulation and could use this to produce several clutches (Rojas *et al*. 2015).

The presence or absence of multiple clutches in considered one of the most fascinating traits thought to vary among the squamate (Ford and Seigel 2006). This phenomenon occurs if the vitellogenesis of a cohort of follicles is initiated while the female is still ovigerous, and there is no period of ovarian quiescence between the consecutive clutches (Mathies 2011). The

evidence of mature follicles and eggs simultaneously suggest that *P. patagoniensis* should be able to produce multiple clutches during the same reproductive season (Shine 1977). This reproductive advantage is allowed due the ability of sperm storage in the utero-vaginal junction. The production of at least two consecutive clutches is now well-documented for a number of Brazilian tropical (Vitt 1983; Marques 1996; Pinto and Fernandes 2004; Pinto *et al*. 2010; Marques and Muriel 2007; Mesquita *et al*. 2011) and subtropical snakes (Balestrin and Di-Berardo 2005; Aguiar and Di-Bernardo 2005; Mesquita *et al*. 2013). *P. patagoniensis* produces multiple clutches per season and because this has extended reproduction. The variable vitellogenesis rates may be related to the income breeding mechanism of vitellogenesis which is mediated by the food resources that is temporally variable (Bonnet *et al*. 1998). Additionally, the seasonality of egg production must coincide with the season that has improved incubation conditions to assure the offspring development, which is not possible in the cooler season (Brown and Shine 2006).

The annual activity pattern of *P. patagoniensis* is influenced by the environmental seasonality, with clear reduction of activity in the cooler months. In South Brazil the rainfall is well-distributed throughout the year, while the seasonal changes of temperature should be the major limiting factor to the snakes activity (Gregory 2009). The subtropical climate of the region resembles the temperate seasonal changes in temperature, and the winter low temperatures impose physiological constraints that limit snakes activity to the warmer months (Balestrin and Di-Bernardo, 2005). However, the activity in snakes may be influenced by the interaction of several another factors like temperature, rainfall, prey availability and reproduction (Shine, 1977; Marques *et al*. 2000). In *P. patagoniensis* the reproductive cycle is an important cause determining the seasonal activity, since males and females presented synchrony in activity peak in spring coinciding with the mating season, and juveniles were more abundant in summer coinciding with the time of births (recruitment). The females activity may be also influenced by the vitellogenic cycle, the thermoregulation behavior during gravidity to enhance egg development, and by the searching for oviposition sites (Shine, 1977). In addition, the life stage can also determine the activity pattern of the snakes being responsible for adult and young activity peaks at different times of the year (Shine 1979). The time of juveniles recruitment occurs during the season of best climatic conditions and greatest abundance of prey enhancing the offspring survival (Shine 1977; Vitt and Caldwell 2014).

Sexual dimorphism in body size has been previously reported to *P. patagoniensis* (Fowler and Salomão 1994; Hartmann and Marques 2005; López and Giraudo 2008) being females larger than males, which is a common pattern in snakes (Shine 1988). The

reproduction-related processes that act on the organisms body size may be distinguished between fecundity selection and sexual selection (Bonnet *et al*. 2000). Body size sexual dimorphism may be correlated to size selection based in fecundity, because larger females produce larger clutches (Shine 1994). Therefore, females invest more in body growth before achieving the sexual maturity. The late sexual maturity advantage is allowing females to reach larger body sizes before starting reproduction. Furthermore, the sexual selection may favor larger females in better body condition, because males are able to distinguish information about condition, age, reproductive state and species based on information provided by female skin lipid pheromones (LeMaster and Mason 2002; Shine *et al*. 2003). To the majority of snake species a female-biased SSD is the rule, while the sexual selection can influences a male-biased SSD in lineages that exhibit pre-copulatory male-male interactions (Shine *et al*. 2000). So, a female-biased SSD is considered an ancestral condition of snakes (Rivas and Burghardt 2000). One the other hand, patterns of female-biased SSD are consistent with the hypothesis that sexual selection favors small male size, which are more efficient in obtain coercive matings (Shine and Mason 2005).

P. patagoniensis larger females (BM and SVL) produces follicles and eggs in greater amount and size. The reproductive effort (GSI) was also associated with female body length (SVL). The maternal body size was directly related to the fecundity with greater females producing larger clutches than their smaller conspecifics (Shine 1994). This can be explaining because larger females have greater abdominal volume to accommodate the eggs (Brown and Shine 2005). The offspring in snakes is mother size-dependent, but in *P. patagoniensis* both fecundity and clutch size ranges widely indicating that are many another factors involved in the reproductive investment (Ford and Seigel 2011). Although, it is difficult to determine the degree of offspring variation influenced by the genotype and/or the environment.

P. patagoniensis exhibits a higher potential fecundity (secondary follicles) compared to the realized/observed fecundity (eggs). Vitellogenesis may represent the major reproductive investment in females and producing more follicles/eggs results in a reduction in follicles/eggs size. This may be explaining because females have control to determine an optimal clutch size-number that provides a higher maternal fitness, seeing the finite energy stores or abdominal volume available for the clutch (Brown and Shine 2009). Considering this, the major reproductive cost is due to the substantial expenditure of energy, mainly in snake species that produce large clutches (Lourdais *et al*. 2003). The other reproduction charge is the trade-off "reproductive investment vs. maternal fitness", where an increase in the reproductive investment negatively affect other components of fitness such as the female survival, the growth

rates and the future reproductive output (Shine and Schwarzkopf 1992; Brown and Weatherhead 2004). Another trade-off related is "maternal fitness vs. offspring fitness|", because it is expected that larger neonates should have several survival advantages: increased locomotion ability to escape predators, shorter time to sexual maturity and enhanced foraging success (Kissner and Weatherhead 2005). However, in oviparous snakes, the neonate size (SVL and/or mass) can be influenced not only by the initial egg mass, but also by the incubation period and egg mass gain during incubation (Brown and Shine 2005).

Therefore, *P. patagoniensis* females have an extended seasonal pattern of reproduction in subtropical Brazil. The seasonal variation follicles/eggs, GSI and shell glands allowed to inferring a reproductive peak. While, some reproductive aspects like sperm storage and producing multiple clutches require more attention, principally analyzing trough the phylogenetic perspective. To complete understand the female reproductive traits and patterns, more field and experimental studies are required, to explain questions about sex steroids and environmental cues, for example. The knowledge about subtropical squamates reproduction should be enlarged with this study, although many further opportunities are still available.

REFERENCES

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

Aldridge, R. D. 1992. Oviductal anatomy and seasonal sperm storage in the Southeastern crowned snake (*Tantilla coronata*). *Copeia* 1992(4):1103-1106.

Aldridge, R. D.; Goldberg, S. R.; Wisniewski, S. S.; Bufalino, A. P. and Dillman, C. B. 2009. The reproductive cycle and estrus in the colubrid snakes of temperate North America. *Contemporary Herpetology* 2009(4):1-31.

Almeida-Santos, S. M. and Salomão, M. G. 1997. Long-Term Sperm Storage in the Female Neotropical Rattlesnake *Crotalus durissus terrrfficus*. *Japanese Journal of Herpetology* 17(2):46-52.

Almeida-Santos, S. M.; Braz, H. B.; Santos, L. C.; Sueiro, L. R.; Barros, V. A.; Rojas, C. A. and Kasperoviczus, K. N. 2014. Biologia reprodutiva de serpentes: recomendações para a coleta e análise de dados. *Herpetologia Brasileira* 3(1):14-24.

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

Balestrin, R. L. and Di-Bernardo, M. 2005. Reproductive biology of *Atractus reticulatus* (Boulenger, 1885) (Serpentes, Colubridae) in Southern Brazil. *Herpetological Journal* 15: 195-199.

Barros, V. A.; Sueiro, L. R. and Almeida-Santos, S. M. 2012. Reproductive biology of the neotropical rattlesnake *Crotalus durissus* from northeastern Brazil: A test of phylogenetic conservatism of reproductive patterns. *Herpetological Journal* 22:97-104.

Birkhead, T. R. and Moller, A. P. 1993. Sexual selection and the temporal separation of reproductive events: sperm storage data from reptiles, birds and mammals. Biological *Journal of the Linnean Society* 50:295-311.

Bonnet, X.; Bradshaw, D. and Shine, R. 1998. Capital versus income breeding: an ectothermic perspective. *Oikos* 83:333-342

Bonnet, X.; Naulleau, G.; Shine, R. and Lourdais. O. 2000. Reproductive versus ecological advantages to larger body size in female snakes, *Vipera aspis*. *Oikos* 89:509-518.

Brown, G. P. and Weatherhead, P. J. 2004. Sexual abstinence and the cost of reproduction in adult male water snakes, *Nerodia sipedon*. *Oikos* 104:269-276.

Brown, G. P. and Shine, R. 2005. Female phenotype, life history and reproductive success in free-ranging snakes (*Tropidonophis mairii*). *Ecology* 86(10): 2763-2770.

Brown, G. P. and Shine, R. 2006. Why do most tropical animals reproduce seasonally? Testing hypotheses on an Australian snake. *Ecology* 87:133-143.

Brown, G. P. and Shine, R. 2009. Beyond size-number tradeoffs: clutch size as a maternal effect. *Philosophical Transactions of the Royal Society B* 364:1097-1106.

Carl Zeiss Microscopy GmbH. 2014. Zen lite for Windows, version 2.

Crews, D. 1984. Gamete production, sex hormone secretion, and mating behavior uncoupled. *Hormonal Behavior* 18:22-28.

Fabian, D. and Flatt, T. 2012. Life History Evolution. *Nature Education Knowledge* 3(8):24.

Ford, N. B. and Seigel, R. A. 1989. Relationships among body size, clutch size, and egg size in three species of oviparous snakes. *Herpetologica* 45:75-83.

Ford, N. B. and Seigel, R. A. 2006. Intra-individual variation in clutch and offspring size in an oviparous snake. *Journal of Zoology* 268:171-176.

Ford, N. B. and Seigel, R. A. 2011. Offspring size variation in snakes. In Aldridge R. D.; Sever D. M. (Eds): *Reproductive Biology and Phylogeny of Snakes*, 573-586. Science Publishers, Enfield.

Ford, N. B. and Seigel, R. A. 2015. The influence of female body size and shape on the trade-off between offspring number and offspring size in two viviparous snakes. *Journal of Zoology* 295:154-158.

Fowler, I. R. and Salomão, M. G. 1994. A study of sexual dimorphism in six species from the colubrid snake genus *Philodryas. The Snake* 26:117-122,

Fowler, I. R.; Salomão, M. G. and Jordão, R. S. 1998. A description of the female reproductive cycle in four species from the neotropical colubrid snake *Philodryas* (Colubridae. Xenodontinae). *The Snake* 28:71-78.

Fox, W. 1956. Seminal receptacles of snakes. *Anatomical Record* 124:519-540.

Gibbons, J. W. and Semlitsch, R. D. 1987. Activity patterns. In Seigel, R. A.; Collins, J. T. and Novak, S. S. (Eds): *Snakes: ecology and evolutionary biology*, 184-209. McGrawHill, New York.

Gregory, P. T. 2009. Northern Lights and Seasonal Sex: The Reproductive Ecology of Cool-Climate Snakes. *Herpetologica* 65(1):1-13.

Hartmann, P. and Marques, O. A. V. 2005. Diet and habitat use of two sympatric species of *Philodryas* (Colubridae) in South Brazil. *Amphibia-Reptilia* 26:25-31.

Hoffman, L. H. 1970. Placentation in the garter snake, *Thamnophis sirtalis. Journal of Morphology* 131:57-88.

Hoffman, L. H. and Wimsatt, W. A. 1972. Histochemical and electron microscopic observations on the sperm receptacles in the garter snake oviduct. *American Journal of Anatomy* 134:71-96.

Kissner, K. J. and Weatherhead, P. J. 2005. Phenotypic effects on survival of neonatal northern watersnakes *Nerodia sipedon. Ecology* 74:259-265.

LeMaster, M. and Mason, R. 2002. Variation in a female sexual attractiveness pheromone controls male mate choice in garter snakes. *Journal of Chemical Ecology* 28:1269-1285.

Loebens, L.; Cechin, S. Z.; Theis, T. F.; Mouras, L. B. and Almeida-Santos, S. M. (in press). Reproductive biology of the *Philodryas patagoniensis* (Snakes: Dipsadidae) in South Brazil: male reproductive cycle.

López, M. S. and Giraudo, A. R. 2008. Ecology of the Snake *Philodryas patagoniensis* (Serpentes, Colubridae) from Northeast Argentina. *Journal of Herpetology* 42(3):474-480.

Lourdais, O.; Bonnet, X.; Shine, R. and Taylor, E. N. 2003. When does a reproducing female viper (*Vipera berus*) "decide" on her litter size? *Journal of Zoology* (London) 259:123-129.

Mathies T. 2011. Reproductive cycles of tropical snakes. In Aldridge R. D.; Sever D. M. (Eds): *Reproductive Biology and Phylogeny of Snakes*, 511-550. Science Publishers, Enfield.

Marques, O. A. V. 1996. Biologia reprodutiva da cobra-coral *Erythrolamprus aesculapii* Linnaeus (Colubridae), no sudeste do Brasil. *Revista Brasileira de Zoologia* 13:747-753.

Marques, O. A. V.; Eterovic, A. and Endo, W. 2000. Seasonal activity of snakes in the Atlantic forest in Southeastern Brazil. *Amphibia-Reptilia* 20:103-111.

Marques, O. A.V. and Muriel, A. P. 2007. Reproductive biology and food habits of the swamp racer *Mastigodryas bifossatus* from Southeastern South America. *Herpetological Journal* 17:104-109.

Mesquita, P. C. M. D.; Borges-Nojosa, D. M.; Passos, D. C. and Bezerra, C. H. 2011. Ecology of *Philodryas nattereri* in the Brazilian semi-arid region. *Herpetological Journal* 21:193-198.

Mesquita, P. C. M. D., Sá-Polidoro, G. L. and Cechin, S. Z. 2013. Reproductive biology of *Philodryas olfersii* (Serpentes, Dipsadidae) in a subtropical region of Brazil. *Herpetological Journal* 23:39-44

Naulleau, G. and Bonnet, X. 1996. Body condition threshold for breeding in a viviparous snake. *Oecologia* 107:301-306.

Oliveira, R. B.; Pontes, G. M. F.; Maciel, A. P.; Gomes, L. R. and Di-Bernardo, M. 2011. Reproduction of *Xenodon dorbignyi* on the north coast of Rio Grande do Sul, Brazil. *Herpetological Journal* 21:219-225.

Perkins, J. M. and Palmer, B. D. 1996. Histology and functional morphology of the oviduct of an oviparous snake, *Diadophis punctatus. Journal of Morphology* 227:67-79.

Peters, J. A. and B. Orejas-Miranda. 1970. Catalogue of the neotropical Squamata. Part I - Snakes. *United States National Museum Bulletin* 297:1-347.

Pinto, R. R. and Fernandes, R. 2004. Reproductive biology and diet of *Liophis poecilogyrus poecilogyrus* (Serpentes, Colubridae) from Southeastern Brazil. *Phyllomedusa* 3:9-14.

Pinto, R. R. and Marques, O. A. V.; Fernandes, R. 2010. Reproductive biology of two sympatric colubrid snakes, *Chironius flavolineatus* and *Chironius quadricarinatus*, from the Brazilian Cerrado domain. *Amphibia-Reptilia* 31:463-473.

Pontes, G. M. F. 2007. História natural de *Philodryas patagoniensis* (Serpentes: Colubridae) no litoral do Rio Grande do Sul, Brasil. PhD Thesis. Pontífica Universidade Católica, Porto Alegre, Rio Grande do Sul, Brazil,

Pontes, G. M. F.; Oliveira, R. B.; Di-Bernardo, M. and Borges-Martins, M. 2003. *Philodryas patagoniensis*. Cannibalism. *Herpetological. Review* 34(2):154.

Rahn, H. 1940. Sperm viability in the uterus of the garter snake, *Thamnophis. Copeia* 1940(2):107-115.

Rivas, J. A. and Burghardt, G. M. 2000. Understanding sexual size dimorphism in snakes: wearing the snake's shoes. *Animal Behaviour* 62:1-6.

Rojas, C. A. 2013. Padrões de estocagem de esperma e variações cíclicas ovidutais em serpentes Xenodontinae. PhD Thesis. Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil.

Rojas, C. A.; Barros, V. A. and Almeida-Santos, S. M. 2015. Sperm storage and morphofunctional bases of the female reproductive tract of the snake *Philodryas patagoniensis* from southeastern Brazil. *Zoomorphology* 134:1-10.

Schuett, G. W. 1992. Is Long-term Sperm Storage an Important Component of the Reproductive Biology of Temperate Pitvipers? In Campbell, J. A.; Brodie, E. D. (Eds): *Biology of Pitvipers*, 169-184. Selva, Texas.

Sever, D. M. and Ryan, T. J. 1999. Ultrastructure of the reproductive system of the black swamp snake (*Seminatrix pygaea*). I. Evidence for oviducal sperm storage. Journal of *Morphology* 241:1-18.

Sever, D. M.; Ryan, T. J.; Morris, T.; Patton, D. and Swafford, S. 2000. Ultrastructure of the Reproductive System of the Black Swamp Snake (*Seminatrix pygaea*). II. Annual Oviducal Cycle. *Journal of Morphology* 245:146-160.

Sever, D. and Hamlett, W. 2002. Female Sperm Storage in Reptiles. *Journal of Experimental Zoology* 187:187-199.

Seigel, R. A. and Ford, N. B. 2001. Phenotypic plasticity in reproductive traits: geographical variation in plasticity in a viviparous snake. *Functional Ecology* 15:36-42.

Shine, R. 1977. Reproduction in Australian elapid snakes. II. Female reproductive cycles. *Australian Journal of Zoology* 25:655-666.

Shine, R. 1979. Activity Patterns in Australian Elapid Snakes (Squamata: Serpentes: Elapidae). *Herpetologica*, 35(1):1-11.

Shine, R. 1988. The Evolution of Large Body Size in Females: A Critique of Darwin's "Fecundity Advantage" Model. *The American Naturalist* 131(1):124-131.

Shine, R. 1994. Sexual size dimorphism in snakes revisited. *Copeia* 1994(2):326-346.

Shine, R. and Schwarzkopf, L. 1992. The Evolution of Reproductive Effort in Lizards and Snakes. *Evolution* 46(1):62-75.

Shine, R.; Olsson, M. M.; Moore, I. and LeMaster, M. P.; Greene, M. and Mason, R. T. 2000. Body size enhances mating success in male garter snakes. *Animal Behaviour* 59:4-11.

Shine, R.; Phillips, B.; Waye, H.; LeMaster, M. and Mason, R. 2003. Chemosensory cues allow courting male garter snakes to assess body length and body condition of potential mates. *Behavioral Ecology and Sociobiology* 54:162-166.

Shine, R. and Mason, R. T. 2005. Does large body size in males evolve to facilitate forcible insemination? A study on garter snakes. *Evolution* 59(11):2426-2432.

Shine, R. and Bonnet, X. 2000. Snakes: a new 'model organism' in ecological research? *Tree* 15(6):221-222.

Shine, R. and Bonnet, X. 2009. Reproductive biology, population viability, and options for field management. In Mullin, S. J.: Seigel, R. A. (Eds): *Snakes Ecology and Conservation*, 172-200. Cornell University Press, Ithaca.

Siegel, D. S. and Sever, D. M. 2008. Seasonal variation in the oviduct of female *Agkistrodon piscivorous* (Reptilia: Squamata): An ultrastructural investigation. *Journal of Morphology* 296:980-997.

Siegel D. S.; Miralles, A.; Chabarria, R. E. and Aldridge R. D. 2011. Female Reproductive Anatomy: Cloaca, Oviduct, and Sperm Storage. In Aldridge R. D.; Sever D. M. (Eds): *Reproductive Biology and Phylogeny of Snakes*, 347-409. Science Publishers, Enfield.

Statsoft, Inc. 2011.Statistica for Windows (data analysis software system), version 10.

Stearns, S. C.; Ackermann, M.; Doebeli, M. and Kaiser, M. 2000. Experimental evolution of aging, growth, and reproduction in fruitflies. *Proceedings of the National Academy of Sciences USA* 97:3309-3313

Systat, Inc. 2011.Sigmaplot for Windows, version 12.0. Systat Software.

Taylor E. N. and DeNardo, D. F. 2011 Hormones and Reproductive Cycles in Snakes. In: Hormones and Reproduction of Vertebrates – Reptiles: 355-372. Norris D.; Lopez (ed.). San Diego: Academic Press.

Torello-Viera, N. F.; Araújo, D. P. and Braz, H. B. 2012. Annual and daily activity patterns of the snail-eating snake *Dipsas bucephala* (Serpentes, Dipsadidae) in southeastern Brazil. *South American Journal of Herpetology* 7(3):252-258.

Tryon, B. W. 1984. Additional Instances of Multiple Egg-Clutch Production in Snakes. *Transactions of the Kansas Academy of Science* 87(3/4):98-104.

Vitt, L. J. 1983. Ecology of an anuran-eating guild of terrestrial tropical snakes. *Herpetologica*, 39: 52-66.

Vitt, L. J. and Caldwell, J. P. 2014. Herpetology: An Introductory Biology of Amphibians and Reptiles, 4th ed., pp. 734. Academic Press, San Diego.

Zar J. R. 1999. Biostatistical Analysis, pp. 875. New Jersey: Prentice-Hall.

Appendix 1. Voucher specimens of *P. patagoniensis* analyzed in this study housed in the herpetological collections of the Federal University of Rio Grande do Sul (UFRGS), Federal University of Santa Maria (ZUFSM) and Pontifical Catholic University of Rio Grande do Sul (MCP-PUCRS)

Macroscopic and anatomical analysis (n=112): ZUFSM0118, 0146, 0261, 0262, 0331, 0342, 0360, 0388, 0399, 0433, 0550, 0652, 0666, 0681, 0684, 0708, 0723, 0866, 0967, 0971, 1088, 1089, 1109, 1130, 1144, 1156, 1171, 1295, 1398, 1404, 1405, 1441, 1650, 1744, 1801, 1855, 1935, 2056, 2089, 2169, 2336, 2431, 2451, 2452, 2476, 2504, 2538, 2581, 2640, 2670, 2696, 2698, 2718, 2720, 2761, 2771, 2986, 3107, and 3112. MCP05257, 05774, 06059, 06158, 12712, 12782, 12793, 12794, 13184, 13185, 13305, 13306, 13411, 13894, 14118, 14325, 14513, 14567, 14568, 14650, 14858, 14995, 15732, 15924, 16144, 16960, 16965, 16966, 16968, 16972, 16978, 16979, 16980, 16981, 16982, 16984, 17013, 17014, 17015, 17016, 17017, 17019, 17021, 17022, 17795, 17837, 17858, 17972, 18133, 18286, 18858 and 19054.

Histology (n=55): ZUFSM0118, 0146, 0262, 0331, 0342, 0360, 0388, 0399, 0433, 0666, 0708, 0866, 0967, 0971, 1088, 1089, 1109, 1130, 1144, 1156, 1295, 1398, 1404, 1441, 1650, 1744, 1801, 1855, 2056, 2431, 2476, 2581, 2718, 2761, 2771, and 3107. MCP12793, 12794, 14567, 16960, 16966, 16968, 16972, 16979, 16981, 16982, 17013, 17016, 17017, 17021, 17022, 17837, 18286, 18858, and 19054.

Seasonal activity (n=783): MCP00159, 00160, 00161, 00163, 00164, 00218, 00254, 00266, 00397, 00545, 00547, 00719, 00755, 00756, 00794, 00831, 00845, 00846, 00847, 00864, 00957, 00977, 01095, 01099, 01105, 01106, 01107, 01184, 01209, 01216, 01217, 01250, 01258, 01259, 01260, 01271, 01400, 01447, 01590, 01749, 01800, 01802, 01867, 01955, 02069, 02079, 02098, 02201, 02284, 02336, 02387, 02397, 02398, 02429, 02503, 02554, 02579, 02581, 02616, 02636, 02730, 02754, 02757, 02832, 02833, 02858, 02873, 02992, 03037, 03048, 03203, 03261, 03336, 03645, 03699, 03701, 03728, 03734, 03844, 03878, 03988, 04011, 04130, 04294, 04295, 04306, 04371, 04463, 04527, 04571, 04578, 04852, 04901, 04963, 05108, 05266, 05267, 05268, 05269, 05270, 05271, 05272, 05273, 05274, 05275, 05277, 05278, 05279, 05280, 05396, 05412, 05413, 05495, 05496, 05498, 05499, 05503, 05504, 05505, 05506, 05507, 05508, 05509, 05510, 05511, 05512, 05612, 05633, 05634, 05635, 05636, 05637, 05638, 05753, 05759, 05774, 05776, 05795, 05843, 05844, 05877, 05953, 05954, 06059, 06060, 06158, 06315, 06331, 06392, 06436, 06450, 06474, 06539, 06540, 06541, 06573, 06574, 06575, 06576, 06577, 06578, 06579, 06830, 06831, 06832, 06833, 06834, 06835, 06836, 06837, 06838, 06839, 06840, 06841, 06842, 06843, 06844, 06943, 06969, 06970, 07003, 07005, 07035, 07115, 07129, 07311, 07312, 07388, 07433, 07434, 07435, 07491, 07739, 07746, 07762, 07783, 07788, 07789, 07790, 07791, 07810, 07830, 07838, 07989, 07997, 08396, 08397, 08398, 08399, 08509, 08522, 08525, 08659, 08660, 08661, 08662, 08663, 08813, 08846, 08954, 08977, 09104, 09105, 09120, 09157, 09162, 09169, 09173, 09174, 09175, 09176, 09345, 09414, 09492, 09907, 09908, 09911, 09912, 09913, 09938, 10527, 10528, 10537, 10538, 10571, 10636, 10637, 10654, 10657, 10674, 10692, 10694, 10767, 10777, 10896, 10898, 10899, 10940, 10948, 10960, 10970, 10971, 10992, 10999, 11043, 11090, 11091, 11115, 11128, 11131, 11135, 11216, 11217, 11218, 11284, 11285, 11310, 11337, 11376, 11414, 11426, 11451, 11467, 11506, 11507, 11508, 11533, 11534, 11535, 11712, 11825, 11848, 11851, 11857, 11908, 11918, 12026, 12044, 12054, 12055, 12095, 12170, 12184, 12186, 12304, 12372, 12487, 12519, 12525, 12528, 12552, 12559, 12577, 12579, 12633, 12653, 12675, 12683, 12688, 12712, 12713, 12747, 12748, 12753, 12782, 12793, 12794, 12795, 12813, 12869, 12904, 13184, 13185, 13186, 13187, 13205, 13233, 13234, 13235, 13270, 13271, 13278, 13296, 13305,

13306, 13411, 13586, 13648, 13790, 13802, 13816, 13817, 13834, 13837, 13838, 13894, 13896, 13898, 13899, 13900, 13971, 14118, 14166, 14178, 14189, 14190, 14191, 14192, 14193, 14194, 14195, 14196, 14197, 14198, 14199, 14200, 14201, 14202, 14203, 14204, 14205, 14206, 14207, 14208, 14209, 14210, 14211, 14212, 14213, 14215, 14216, 14217, 14218, 14219, 14220, 14221, 14222, 14223, 14224, 14228, 14239, 14246, 14263, 14268, 14271, 14277, 14296, 14325, 14336, 14352, 14389, 14390, 14394, 14468, 14482, 14513, 14522, 14530, 14567, 14568, 14569, 14570, 14631, 14636, 14650, 14660, 14703, 14706, 14714, 14717, 14718, 14774, 14850, 14858, 14878, 14880, 14897, 14995, 15001, 15002, 15448, 15512, 15515, 15516, 15679, 15680, 15681, 15682, 15683, 15684, 15711, 15712, 15727, 15728, 15729, 15730, 15731, 15732, 15733, 15734, 15756, 15846, 15847, 15924, 15926, 15927, 15928, 15990, 16143, 16144, 16145, 16146, 16147, 16148, 16149, 16722, 16723, 16788, 16789, 16944, 16945, 16946, 16947, 16948, 16949, 16950, 16951, 16952, 16953, 16954, 16955, 16956, 16957, 16958, 16960, 16961, 16962, 16963, 16964, 16965, 16966, 16967, 16968, 16969, 16970, 16971, 16972, 16973, 16974, 16975, 16978, 16979, 16980, 16981, 16982, 16983, 16984, 16985, 17012, 17013, 17014, 17015, 17016, 17017, 17018, 17019, 17020, 17021, 17022, 17024, 17458, 17713, 17719, 17740, 17795, 17837, 17856, 17857, 17858, 17859, 17894, 17955, 17966, 17972, 17988, 17989, 17999, 18065, 18066, 18067, 18133, 18165, 18283, 18286, 18367, 18368, 18369, 18444, 18469, 18472, 18478, 18487, 18496, 18501, 18613, 18697, 18719, 18726, 18727, 18821, 18835, 18858, 18901, 18909, 18954, 18973, 18974, 19054, 19089, 19102, 19110, 19111, 19133, 19154, and 19215; UFGRS1055, 1063, 1064, 1065, 1066, 1067, 1068, 1069, 1070, 1072, 1073, 1074, 1075, 1077, 1078, 1085, 1088, 1238, 1239, 1240, 1241, 1733, 2067, 2110, 2316, 2816, 2858, 3382, 3561, 3660, 4014, 4164, 4558, 4803, 4804, 4967, 5150, 5151, 5235, 5941, 6016, 6147, 6347, 6355, 6356, and 6759; ZUFSM0048, 0117, 0118, 0146, 0261, 0262, 0331, 0342, 0352, 0353, 0356, 0360, 0381, 0384, 0388, 0390, 0391, 0399, 0426, 0433, 0482, 0491, 0534, 0543, 0550, 0639, 0640, 0644, 0649, 0652, 0666, 0681, 0684, 0689, 0696, 0708, 0723, 0726, 0738, 0781, 0794, 0795, 0835, 0842, 0866, 0868, 0880, 0904, 0905, 0906, 0907, 0908, 0909, 0961, 0967, 0968, 0971, 0978, 0990, 1017, 1088, 1089, 1109, 1117, 1130, 1144, 1156, 1171, 1251, 1262, 1263, 1295, 1303, 1398, 1404, 1405, 1419, 1430, 1435, 1437, 1441, 1455, 1459, 1460, 1461, 1462, 1463, 1464, 1465, 1466, 1601, 1608, 1650, 1675, 1684, 1690, 1703, 1718, 1725, 1744, 1748, 1749,1750, 1751, 1752, 1753, 1801, 1853, 1855, 1935, 1971, 1979, 2056, 2089, 2169, 2175, 2229, 2293, 2295, 2296, 2297, 2298, 2299, 2300, 2302, 2303, 2304, 2305, 2326, 2347, 2350, 2427, 2429, 2430, 2431, 2451, 2452, 2470, 2476, 2504, 2515, 2538, 2566, 2577, 2581, 2588, 2604, 2633, 2640, 2670, 2696, 2698, 2718, 2720, 2737, 2743, 2744, 2746, 2761, 2766, 2771, 2896, 2913, 2986, 2997, 3004, 3021, 3041, 3051, 3098, 3107, 3112, 3176, 3190, 3193, 3203, 3214, 3225, 3234, 3249, 3250, 3257, 3259, and 3270.

TABLES AND FIGURES

Table 1: Macroscopic and microscopic measurements for females of *P. patagoniensis* in South Brazil. Post hoc analysis: significant differences (p<0.05) between seasons are indicated by letters. Each letter represents a season. Date is expressed as mean ± standard errors. SVL: snout-vent length; GSI: gonadosomatic index.

Season	SVL (mm)	GSI	Follicles diameter (mm)	Shell glands (µm)
Summer (A)	844.93±39.72	0.57±0.09 [C, D]	8.44±1.73 [C, D]	24.51±2.08 [C, D]
Autumn (B)	859.50±29.83	0.73±0.12 [C, D]	5.95±0.37 [C, D]	31.96±4.89
Winter (C)	919.86±53.37	3.54±1.05 [A, B]	12.27±2.09 [A, B]	42.79±4.50 [A]
Spring (D)	851.18±18.25	3.72±0.94 [A, B]	11.73±0.99 [A, B]	43.89±3.25 [A]

Table 2: Phases of the annual reproductive cycle of female *P. patagoniensis* in South Brazil

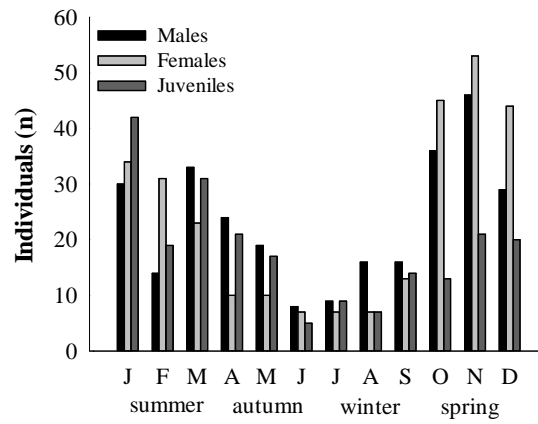|	Summer	Autumn	Winter	Spring
Peak activity of males and females				▓
Peak activity of juveniles	▓			
Secondary vitellogenesis peak			▓	▓
GSI increases			▓	▓
Shell glands hypertrophy			▓	▓
Long-term sperm storage (utero-vaginal junction)	▓		▓	▓
Short-term sperm storage (infundibulum)	▓		▓	▓
Ovulation			▓	▓
Mating	▓	▓		▓
Gravidity	▓		▓	▓
Presence of follicles and eggs	▓			▓
Oviposition	▓			▓
Bitrhs/ hatchling	▓		▓	

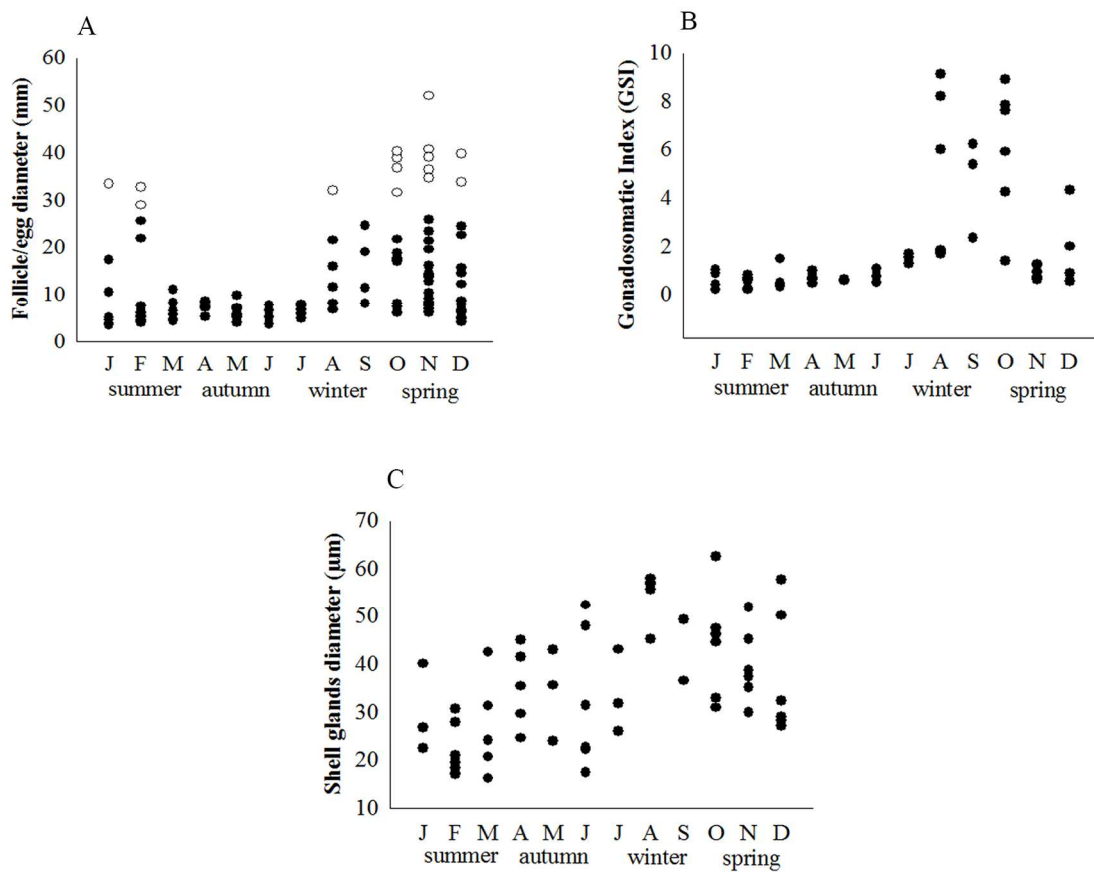Figure 1: Seasonal activity of *P. patagoniensis* in South Brazil.

Figure 2. Seasonal variation in (A) follicles/eggs diameter, (B) gonadosomatic index, and (C) shell glands diameter for females of *P. patagoniensis* in South Brazil. (●): vitellogenic follicles. (○): eggs.
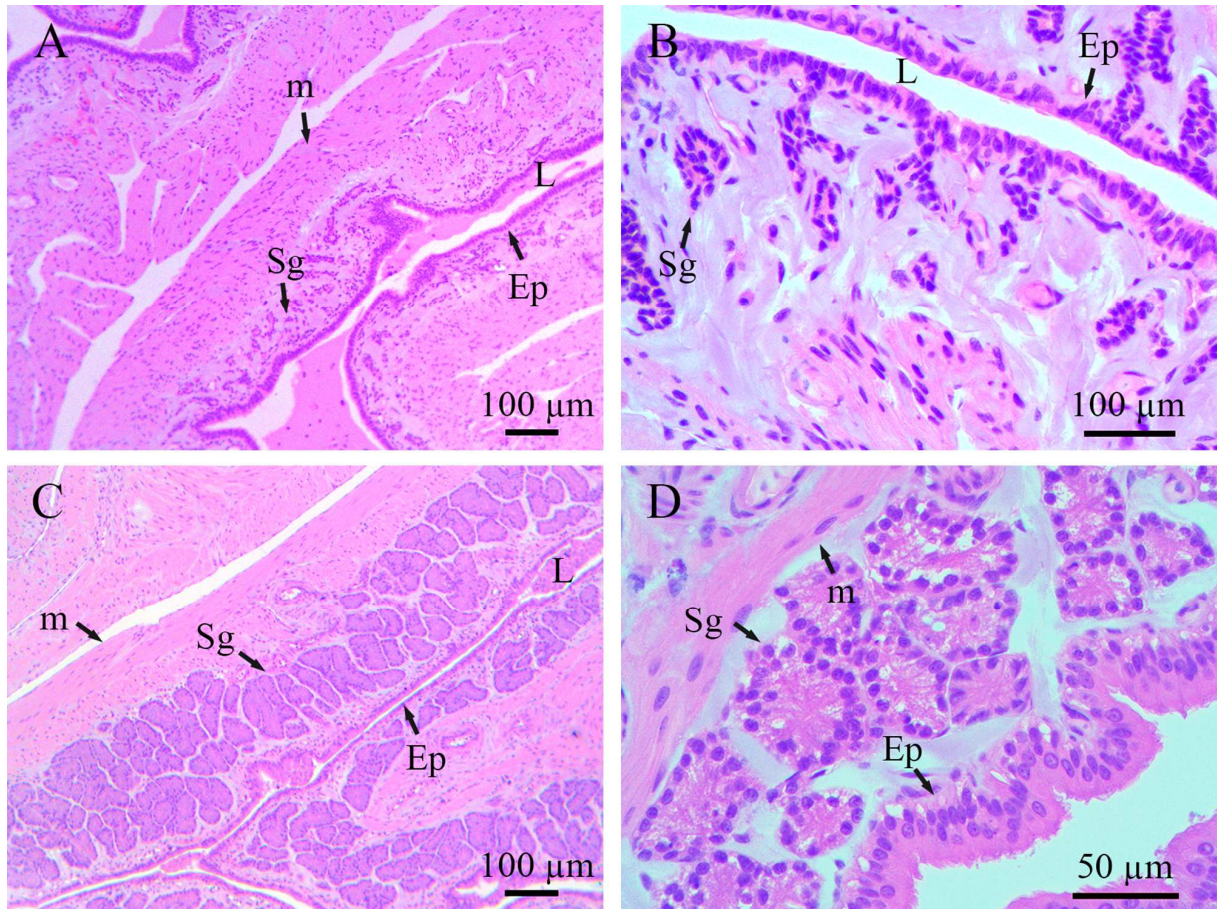
Figure 3. Transverse sections of the uterus of *P. patagoniensis* showing shell glands in atrophy during summer-autumn (A, B), and hypertrophy during winter-spring (C, D). Ep: epithelium; Sg: Shell gland; m: muscularis; L; lumen.
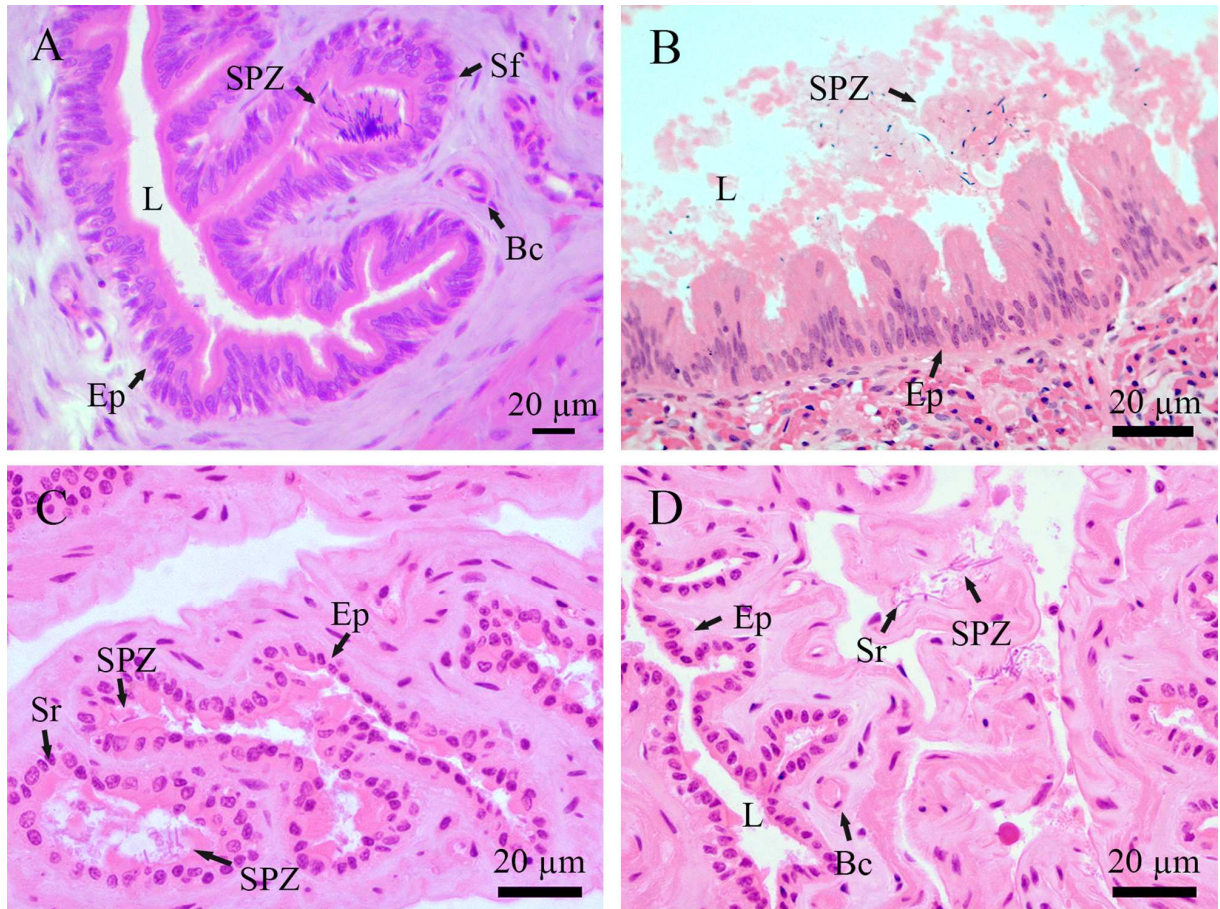
Figure 4. Transverse sections of the utero-vaginal junction of *P. patagoniensis* exhibiting sperm storage in furrows /glands (A), vagina with post-copulating sperm (B) and the posterior infundibulum exhibiting sperm storage receptacles (C e D). Ep: epithelium; Bc blood capillaries; L: lumen; SPZ: spermatozoa; Sf: sperm storage furrows; Sr sperm storage receptacles.
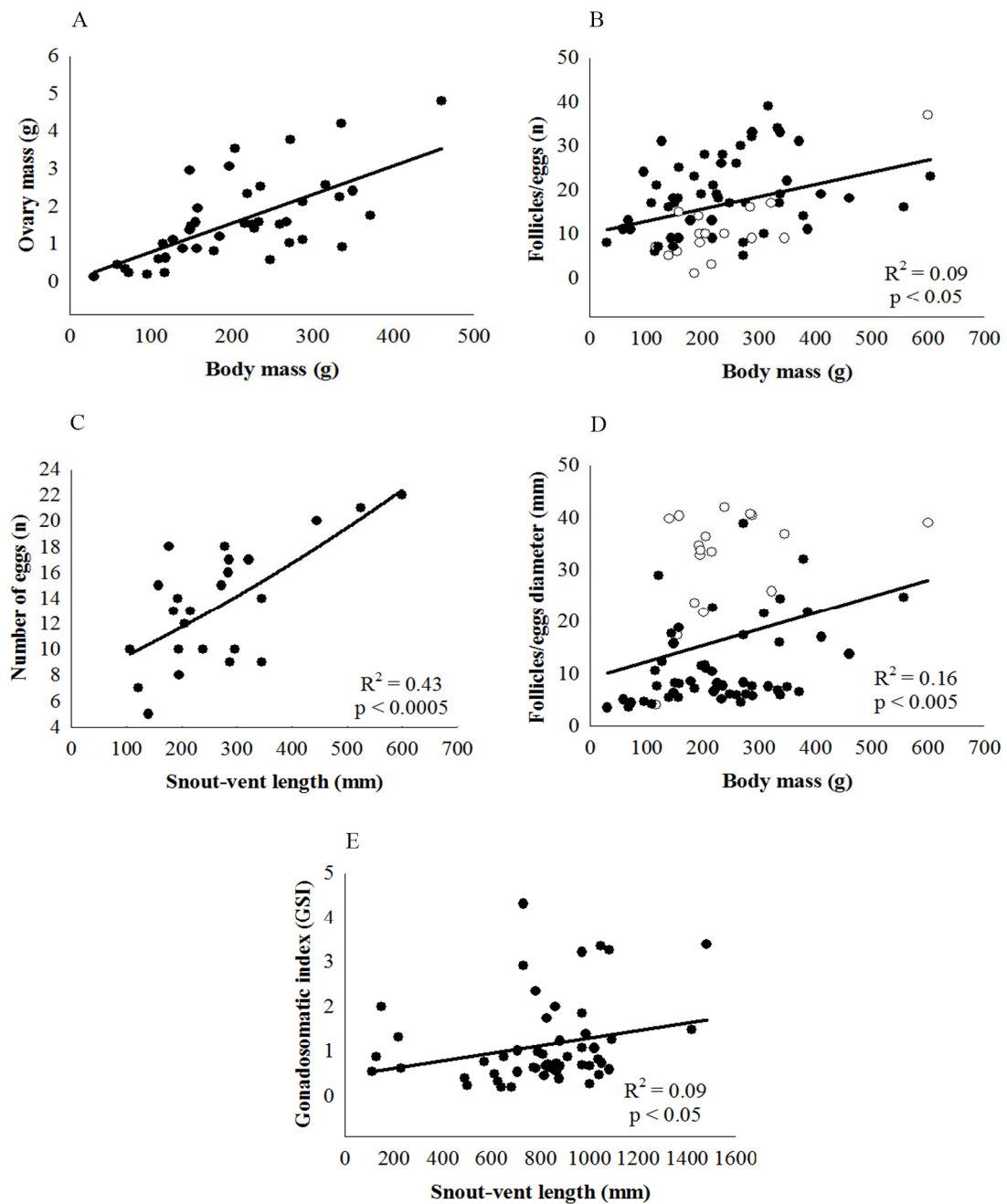
Figure 5. Relationship between (A) ovary mass and body mass (B) number of follicles/eggs and body mass, (C) clutch size and SVL for females, (D) follicles/eggs diameter and body mass, and (E) gonadosomatic index and body size of *P. patagoniensis* in South Brazil. (●): vitellogenic follicles. (○): eggs.
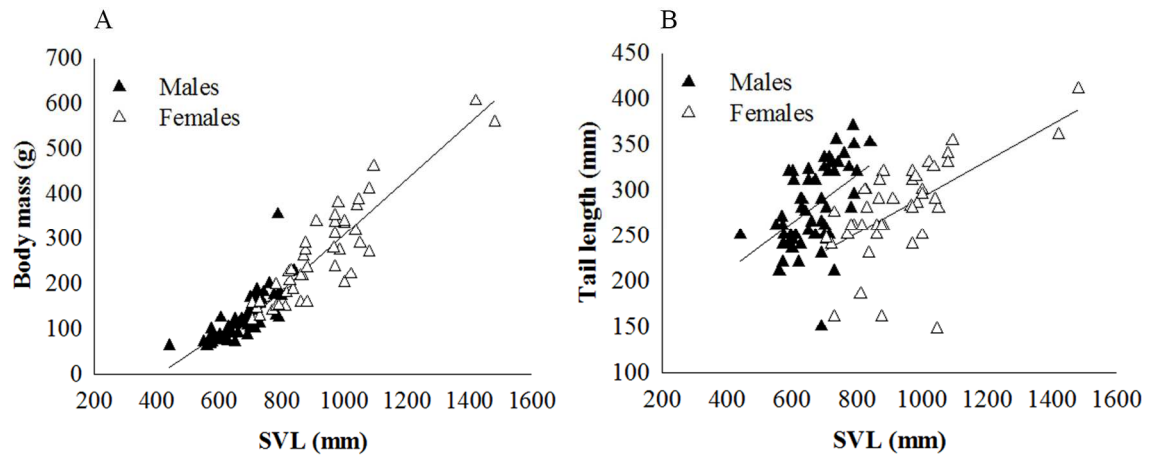
Figure 6. Sexual dimorphism in (A) body size and (B) tail length (C) of *P. patagoniensis* in South Brazil. (▲): males. (△): females.
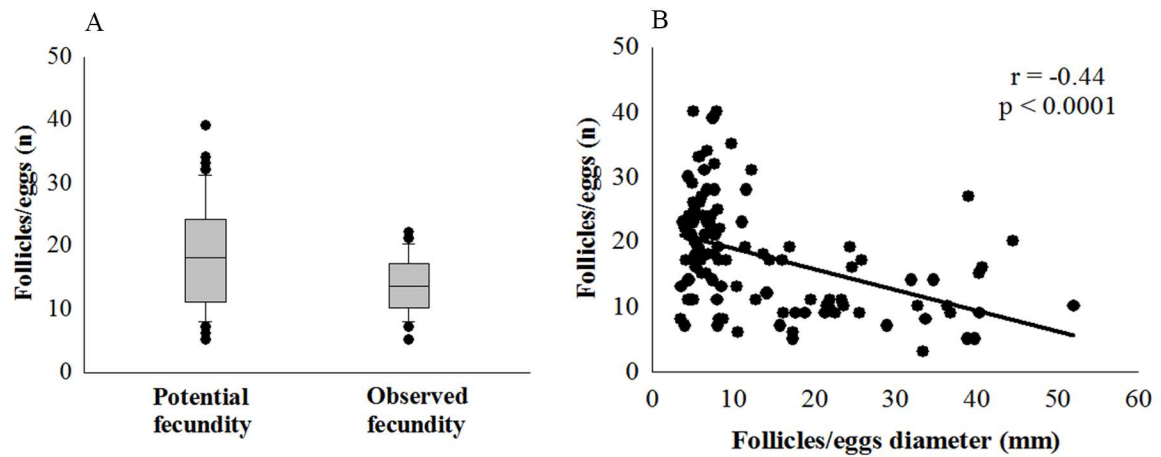

Figure 7. (A) Potential vs. observed fecundity; (B) Relationship between follicles/eggs diameter (size) and number of *P. patagoniensis* in South Brazil. Middle line represents average values, boxes show standard deviations and whiskers represent minimum and maximum values.

CONCLUSÕES

- O ciclo reprodutivo da espécie *P. patagoniensis* na região Sul do Brasil é descrito como sazonal, podendo ser considerado estendido.

- Em nível de indivíduo os machos exibem um ciclo reprodutivo cíclico descontínuo, enquanto a nível populacional, o ciclo reprodutivo é sazonal semi-sincrônico, com a maioria dos indivíduos apresentando pico reprodutivo no período primavera-verão.

- Em nível de indivíduo as fêmeas exibem um ciclo reprodutivo cíclico descontínuo, enquanto a nível populacional o ciclo reprodutivo é sazonal semi-sincrônico. As fêmeas permanecem reprodutivamente ativas durante um longo período do ano, porém o pico da vitelogênese secundária ocorre no período inverno-primavera.

- O pico de atividade sazonal de machos e fêmeas coincide com o período reprodutivo na primavera, enquanto o pico de atividade de juvenis no verão relaciona-se à época de recrutamento.

- O pico de atividade segmento sexual renal precede o período de máxima atividade testicular, demonstrando que o desenvolvimento desta estrutura é energeticamente custoso.

- As fêmeas de *P. patagoniensis* tem capacidade para produzir múltiplas desovas com esperma estocado a longo prazo no útero posterior, de modo que hajam duas épocas de nascimento de filhotes simultâneas.

- *P. patagoniensis* apresenta dimorfismo sexual no tamanho do corpo, sendo as fêmeas significativamente maiores e mais pesadas do que os machos, enquanto os machos apresentam caudas maiores que as fêmeas.

- O tamanho de maturidade sexual é maior para as fêmeas em relação aos machos, já que estas investem mais em crescimento antes de reproduzir.

- As fêmeas maiores são capazes de produzir folículos e ovos em maior quantidade e tamanho, logo observa-se uma relação direta do tamanho corpóreo materno com o esforço reprodutivo e a fecundidade.

- As fêmeas produzem muito mais folículos secundários do que são fecundados, logo a fecundidade observada é menor do que a esperada.