

## Reproductive timing and fecundity in the Neotropical lizard *Enyalius perditus* (Squamata: Leiosauridae)

SERENA NAJARA MIGLIORE<sup>1,2,\*</sup>, HENRIQUE BARTOLOMEU BRAZ<sup>2,3</sup>, ANDRÉ FELIPE BARRETO-LIMA<sup>4</sup>, SELMA MARIA ALMEIDA-SANTOS<sup>1,2</sup>

<sup>1</sup> Setor de Anatomia, Departamento de Cirurgia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Orlando Marques de Paiva 87, Cidade Universitária, São Paulo, SP, Brazil. 05508-270

<sup>2</sup> Laboratório de Ecologia e Evolução, Instituto Butantan - Av. Dr. Vital Brazil, 1500 Butantã, São Paulo - SP, Brazil. 05503-900

<sup>3</sup> School of Life and Environmental Sciences, Heydon-Laurence Building, A08, University of Sydney, NSW, 2006, Australia

<sup>4</sup> Laboratório de Herpetologia, Departamento de Zoologia, Instituto de Ciências Biológicas, Campus Darci Ribeiro, Universidade de Brasília - DF, Brazil. 70910-900

\*Corresponding author. E-mail: serena\_891@hotmail.com

Submitted on: 2016, 16<sup>th</sup> December; revised on: 2017, 24<sup>th</sup> February; accepted on: 2017, 29<sup>th</sup> July  
Editor: Giovanni Scillitani

**Abstract.** *Enyalius perditus* is a semi-arboreal lizard species whose reproduction is poorly known. Here, we combine information obtained from preserved and live specimens to describe the reproductive timing (vitellogenesis, gravidity, and egg-laying) and fecundity (clutch size, egg size, and relative clutch mass) in females of *E. perditus*. Female reproduction is remarkably seasonal and occurs in the warmer and wetter periods of the year. Secondary vitellogenesis occurs from mid to late spring, whereas gravidity and egg-laying occur in early summer. Mating appears to be synchronized with secondary vitellogenesis, indicating an associated reproductive cycle. We suggest that *E. perditus* females produce only a single clutch per reproductive season. Clutch size ranged from three to 11 eggs and was positively correlated with female body size. Finally, the relative clutch mass was high, a recurrent feature to “sit-and-wait” foragers.

**Keywords.** Clutch size, reproductive biology, reproductive cycle, seasonal reproduction.

Detailed information on the reproductive biology of a number of species is critical for elaborating and testing ecological-evolutionary hypotheses and providing informed decisions on conservation strategies (Shine and Bonnet, 2009; Vitt, 2013). Despite the recent increase in the number of studies on the reproduction of Neotropical lizards (e.g., Balestrin et al., 2010; Ferreira et al., 2009; Vieira et al., 2001), knowledge about the reproductive biology of several species is still scarce.

*Enyalius* is composed of insectivorous, diurnal, and semi-arboreal lizards (Jackson, 1978; Rautenberg and Laps, 2010; Sturaro and Silva, 2010; Barreto-Lima and Sousa, 2011; Barreto-Lima et al., 2013). *Enyalius* is distributed mostly in Atlantic Forest areas but some species

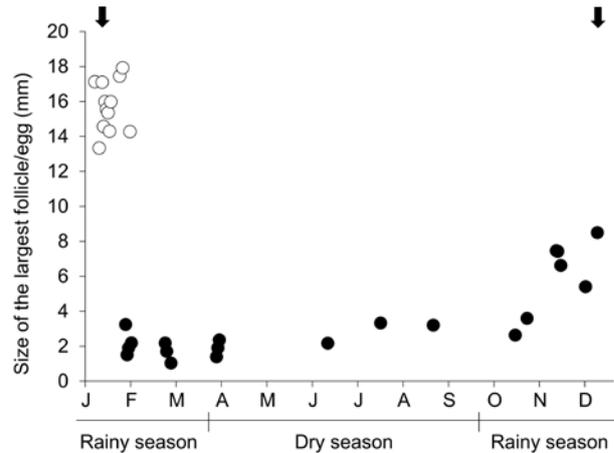
may also occur in the Amazon Forest, forest galleries in ‘Cerrado’, and isolated forested areas in ‘Caatinga’ (Barreto-Lima, 2012).

*Enyalius perditus* is commonly found in Atlantic forest areas in southeastern Brazil, where it may occur in sympatry with *E. brasiliensis*, *E. iheringii*, and *E. bilineatus* (Barreto-Lima, 2012). Many studies have addressed several aspects of the natural history of the species, including feeding ecology, activity patterns, microhabitat use, sexual dimorphism, and behavior (Barreto-Lima and Sousa, 2006, 2011, Sturaro and Silva, 2010; Barreto-Lima et al., 2013; Migliore et al., 2014). Recently, Migliore et al. (2014) summarized the reproductive information available for *E. perditus* and *E. iheringii* and found that published data is

limited to punctual observations on clutch size, courtship and mating behavior, and timing of mating and gravidity. This limited information impairs both an overview on the reproduction of the species and broad comparisons across other species. To fill this gap, we combine information obtained from both museum and live specimens to describe the reproductive timing (vitellogenesis, gravidity, and egg-laying) and fecundity (clutch size, egg size, and relative clutch mass) in females of *E. perditus*.

We analyzed 35 sexually mature females of *E. perditus* housed in six scientific collections from Brazil (Appendix 1). The specimens were mostly collected throughout the Atlantic forest domain. The climate in the area is seasonal and characterized by a distinct hot, rainy season from spring to summer (October-March) and a dry season from autumn to winter (April-September) associated with lower temperatures (Mendonça and Danni-Oliveira, 2007). Females were considered sexually mature if they contained vitellogenic follicles, oviductal eggs, or folded oviducts. For each individual, we recorded the: (1) snout-vent length (SVL) to the nearest 1 mm, (2) number of ovarian follicles or eggs, (3) diameter of the largest ovarian follicle, and (4) length and width of oviductal eggs. Additional observations on body sizes, egg-laying, egg size, and clutch size were obtained from two gravid females collected in the Biological Reserve of Boracéia, São Paulo state, on 8th December 2015. These females were kept in terrarium containing branches and leaf litter and allowed to oviposit naturally. Procedures for egg measurements and incubation were used according to Migliore et al. (2014).

We determined the timing of secondary vitellogenesis using a scatterplot of the diameter of the largest ovarian follicle (Almeida-Santos et al., 2014). Relative clutch mass (RCM) was calculated by dividing the total clutch mass by maternal body mass after oviposition + total clutch mass (Vitt and Price, 1982). We used simple linear regression to determine the relationship between maternal SVL and clutch size (both log-transformed; King, 2000) and significance was assumed at  $P \leq 0.05$ . Mean values are always followed by  $\pm$  standard deviation.



**Fig. 1.** Reproductive timing in females of *Enyalius perditus*. The graph shows the seasonal variation in the diameter of the largest ovarian follicle or oviductal egg and the timing of egg-laying. Closed circle: ovarian follicles; open circle: oviductal eggs; arrows: egg-laying.

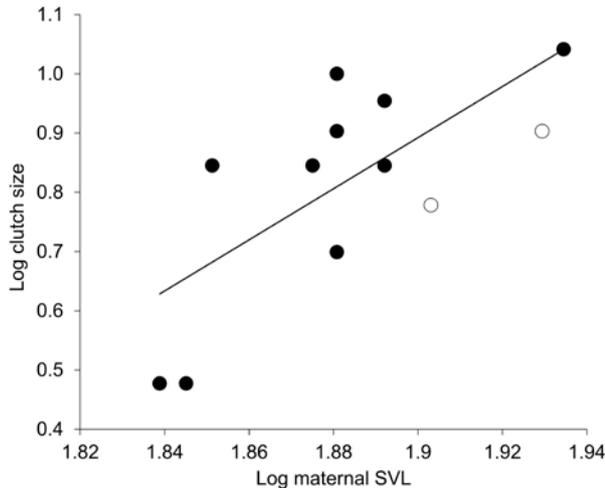
Body sizes of *E. perditus* females ranged from 58 to 91 mm (mean =  $74.7 \pm 7.8$  mm;  $n = 36$ ). Females with follicles in primary vitellogenesis were found throughout the year (Fig. 1). Substantial increases in follicular size (and thus secondary vitellogenesis) were observed from mid to late spring (November-December; Fig. 1). Gravid females ( $n = 11$ ) were observed in early-summer (January; Fig. 1). No female contained follicles in secondary vitellogenesis simultaneously with oviductal eggs. Egg-laying was recorded in early summer (Table 1). The two wild-caught females laid eight and six eggs each on 26th December 2015 and 1st January 2016, respectively (Fig. 1). All eggs spoiled over incubation due to fungal contamination. Clutch size (including preserved and captive specimens) averaged  $7.1 \pm 2.4$  eggs (range: 3-11 eggs;  $n = 13$  clutches). Clutch size was positively correlated with maternal SVL ( $r = 0.70$ ;  $n = 13$ ;  $P = 0.008$ ; Fig. 2). Egg length in all gravid females averaged  $15.45 \pm 1.36$  mm (range: 12.45-17.80 mm;  $n = 92$  eggs from 13 females) and egg width averaged  $8.15 \pm 0.56$  mm (range: 7.38-

**Table 1.** Morphometrics of two clutches of *Enyalius perditus* from Biological Reserve of Boracéia, São Paulo state, Brazil. RCM: Relative clutch mass.

| Individual | Date laid   | Clutch size | Female mass <sup>1</sup><br>(g) | Total clutch<br>mass (g) | RCM <sup>2</sup> | Egg length<br>(mm) | Egg width<br>(mm) | Egg mass<br>(g) |
|------------|-------------|-------------|---------------------------------|--------------------------|------------------|--------------------|-------------------|-----------------|
| Female 1   | 26 Dec 2015 | 8           | 12.3                            | 6.91                     | 0.36             | $16.71 \pm 0.28$   | $9.58 \pm 0.12$   | $0.86 \pm 0.02$ |
| Female 2   | 1 Jan 2016  | 6           | 11.4                            | 4.28                     | 0.27             | $16.09 \pm 0.43$   | $8.66 \pm 0.26$   | $0.71 \pm 0.01$ |

<sup>1</sup>Post partition mass.

<sup>2</sup>RCM was calculated by dividing the total clutch mass by maternal body mass after oviposition plus total clutch mass (Vitt and Price, 1982).



**Fig. 2.** Relationship between maternal snout-vent length and clutch size (both log-transformed) in *Enyalius perditus*. Closed circles: data from preserved specimens; open circles: data from freshly laid clutches.

9.58 mm;  $n = 92$  eggs from 13 females). RCM for the two wild-caught females that laid eggs in captivity was 0.27 and 0.36.

Reproductive timing in *E. perditus* females is remarkably seasonal, with secondary vitellogenesis, gravidity, and egg-laying occurring within three months, from November to January (see Sturaro and Silva, 2010; Barreto-Lima and Sousa, 2011 for additional records of vitellogenesis and gravidity). Therefore, reproductive timing in *E. perditus* females is associated with the warmer and wetter periods of the year. Reproductive information for other *Enyalius* species is rather limited. However, the reproductive timing in *E. perditus* appears to be concentrated within a shorter period of time than other *Enyalius* from the Atlantic forest (Marques and Sazima, 2004; Teixeira et al. 2005; Rautenberg and Laps, 2011; Migliore et al., 2014). This short reproductive season in females of *E. perditus* suggests that females produce only a single clutch per reproductive season. Indeed, this is corroborated by the absence of females containing follicles in secondary vitellogenesis simultaneously with oviductal eggs (Almeida-Santos et al., 2014).

Associated reproductive cycles are common in lizards and consist of reproductive events in males and females (i.e., sperm production, mating, and ovulation) occurring at the same period (Crews and Gans, 1992; Méndez-de la Cruz et al., 2014). Courtship and mating in *E. perditus* have been reported in spring (November–December: Barreto-Lima and Sousa, 2006; Migliore et al., 2014) and thus are synchronized with the timing of secondary vitellogenesis and ovulation. This suggests that *E. perditus*

exhibits associated reproductive cycles. However, histological investigations of the reproductive cycle of *E. perditus* males are required to confirm if the species exhibits associated reproductive cycles.

Mean clutch size in *E. perditus* is low relative to at least three other congeners (14.0 eggs in *E. iheringii*: Rautenberg and Laps, 2010; Migliore et al., 2014; 12.3 eggs in *E. leechi*: Vitt et al., 1996; and 11.5 eggs in *E. brasiliensis*: Teixeira et al., 2005) but high relative to another congeneric (4.4 eggs in *E. bilineatus*: Teixeira et al., 2005). These differences may be explained by interspecific differences in mean body size since all *Enyalius* species that showed higher clutch size than *E. perditus* also exhibited larger body sizes (see Rand, 1982; Vitt et al., 1996; Teixeira et al., 2005; Rautenberg and Laps, 2010). This idea is corroborated by our finding that the clutch size in *E. perditus* increased with maternal SVL, as observed in other *Enyalius* (Teixeira et al., 2005; Vitt et al., 1996) and in many lizard species with variable clutch size (Fitch, 1970; Tinkle et al., 1970).

The RCM for *E. perditus* (0.27–0.36) is similar to that reported for a congeneric (0.38 in *E. iheringii*; Migliore et al., 2014). In lizards, RCM tends to be relatively low in “wide forager” species and relatively high in “sit-and-wait” foragers (Vitt and Price, 1982). Females of *Enyalius* species appear to be “sit-and-wait” foragers (Sousa and Cruz, 2008; Borges et al., 2013) and move shorter distances than males (Barreto-Lima et al., 2013). The RCM for *Enyalius* is higher than the upper limit reported for other oviparous lizards that forage widely ( $\sim 0.21$ ) and consistent with values reported for “sit-and-wait” foragers (Vitt and Price, 1982), thus in agreement with the association between RCM and foraging tactics.

#### ACKNOWLEDGEMENTS

We thank G. Puerto, P. R. Manzani, J. C. Moura-Leite, M. R. S. Pires, R. N. Feio, and V. Silva for allowing access to specimens under their care, M. T. Rodrigues for providing some specimens, M. Teixeira-Junior for assistance in field work. We also thank P. Monteiro, N. Torello-Vieira and K. Banci for criticism on an earlier draft. We thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for providing financial support (Master’s scholarship to S. N. Migliore), SISBIO (Sistema de Autorização e Informação em Biodiversidade; permit number SISBIO 47011-4), and Butantan Institute Animal Ethics Committee (approval number 3491021015) for the permission to collect and maintain the lizard specimens at the Ecology and Evolution Lab–Butantan Institute.

## REFERENCES

- Almeida-Santos, S.M., Braz, H.B., Santos, L.C., Sueiro, L.R., Barros, V.A., Rojas, C.A., Kasperoviczus, K.N. (2014): Biologia reprodutiva de serpentes: recomendações para a coleta e análise de dados. *Herpetol. Bras.* **3**: 14-24.
- Balestrin, R.L., Cappellari, L.H., Outeiral, A.B. (2010): Biologia reprodutiva de *Cercosaura schreibersii* (Squamata, Gymnophthalmidae) e *Cnemidophorus laceroides* (Squamata, Teiidae) no Escudo Sul-Riograndense, Brasil. *Biota Neotrop.* **10**: 131-139.
- Barreto-Lima, A.F. (2012): Distribuição, nicho potencial e ecologia morfológica do gênero *Enyalius* (Squamata Leiosauridae): Testes de hipóteses para lagartos de florestas continentais brasileiras. Unpublished doctoral dissertation. Universidade Federal do Rio Grande do Sul, Porto Alegre.
- Barreto-Lima, A.F., Pires, E.O., Sousa, B.M. (2013): Activity, foraging mode and microhabitat use of *Enyalius perditus* (Squamata) in a disturbed Atlantic rainforest in southeastern Brazil. *Salamandra* **49**: 177-185.
- Barreto-Lima, A.F., Sousa, B.M. (2006): Court and copulation behaviors of *Enyalius perditus* Jackson, 1978 (Squamata, Leiosauridae) in captivity conditions. *Rev. Bras. Zool.* **8**: 193-197.
- Barreto-Lima, A.F., Sousa, B.M. (2011): Feeding ecology and sexual dimorphism of *Enyalius perditus* in an Atlantic forest, Brazil. *Herpetol. Bull.* **118**: 1-9.
- Borges, V.S., Pires, R.C., Linares, A.M., Eterovick, P.C. (2013): Diet of *Enyalius bilineatus* (Leiosauridae: Squamata) at a site in southeastern Brazil: effects of phylogeny and prey availability. *J. Nat. Hist.* **47**: 2785-2794.
- Crews, D., Gans, C. (1992): The interaction of hormones, brain, and behavior: An emerging discipline in herpetology In: *Biology of the Reptilia* 18, pp. 1-23. Gans, C., Crews, D., Eds, The University of Chicago Press, Chicago.
- Ferreira, A.A., Silva, D.N.A., Van Sluys, M.B., Dolder, H.C. (2009): Seasonal changes in testicular and epididymal histology of the tropical lizard, *Tropidurus itambere* (Rodrigues, 1987), during its reproductive cycle. *Brazil. J. Biol.* **69**: 429-435.
- Fitch, H.S. (1970): Reproductive cycles of lizards and snakes. *Misc. publ. Univ. Kans. Mus. Nat. Hist.* **52**: 1-247.
- Jackson, J.F. (1978): Differentiation in the genera *Enyalius* and *Strobilurus* (Iguanidae): implications for Pleistocene climatic changes in eastern Brazil. *Arq. Zool.* **30**: 1-79.
- King, R.B. (2000): Analyzing the relationship between clutch size and female body size in reptiles. *J. Herpetol.* **34**: 148-150.
- Marques, O.A.V., Sazima, I. (2004): História natural dos répteis da Estação Ecológica Juréia-Itatins. In: *História Natural da Estação Ecológica Juréia-Itatins: Ambiente Físico, Flora e Fauna*, p. 257-277. Marques, O.A.V., Duleba, W., Eds, Ribeirão Preto, Holos.
- Méndez-de la Cruz, F. R., Manríquez N. L. M., Ríos, E. A., Iburgüengoytia N. (2014): Male Reproductive Cycles in Lizards In: *Reproductive Biology and Phylogeny of Lizards and Tuatara*, pp. 302-339. Riebel, J., Siegel, D. S., Trauth, S. E., Eds, CRC Press, Enfield.
- Mendonça, F., Danni-Oliveira, I.M. (2007): *Climatologia: Noções Básicas e Climas do Brasil*. Oficina de Textos, São Paulo.
- Migliore, S.N., Braz, H.B., Almeida-Santos, S.M. (2014): Reproductive aspects of two *Enyalius* lizards from the Atlantic forest in Southeastern Brazil. *Herpetol. Notes* **7**: 273-276.
- Rand, A.S. (1982): Clutch and egg size in Brazilian iguanid lizards. *Herpetologica* **38**: 171-178.
- Rautenberg, R., Laps, R.R. (2010): Natural history of the lizard *Enyalius iheringii* (Squamata, Leiosauridae) in southern Brazilian Atlantic forest. *Iheringia, Sér. Zool.* **100**: 287-290.
- Shine, R., Bonnet, X. (2009): Reproductive biology, population viability, and options for field management. In: *Snakes: Ecology and Conservation*, pp. 172-200. Mullin, S.J., Seigel, R.A., Eds, Cornell University Press, Ithaca.
- Sousa, B.M., Cruz, C.A.G. (2008): Hábitos alimentares de *Enyalius perditus* (Squamata, Leiosauridae) no Parque Estadual do Ibitipoca, Minas Gerais, Brasil. *Iheringia, Sér. Zool.* **98**: 260-265.
- Sturaro, M.J., Silva, V.X. (2010): Natural history of the lizard *Enyalius perditus* (Squamata: Leiosauridae) from an Atlantic forest remnant in southeastern Brazil. *J. Nat. Hist.* **44**: 1225-1238.
- Teixeira, R.L., Roldi, K., Vrcibradic, D. (2005): Ecological comparisons between the sympatric lizards *Enyalius bilineatus* and *Enyalius brasiliensis* (Iguanidae, Leiosaurinae) from an Atlantic rain-forest area in Southeastern Brazil. *J. Herpetol.* **39**: 504-509.
- Tinkle, D.W., Wilbur, H.M., Tilley, S. G. (1970): Evolutionary strategies in lizard reproduction. *Evolution* **24**: 55-74.
- Vieira, G.H.C., Wiederhecker, H.C., Colli, G.R. Bão, S. N. (2001): Spermiogenesis and testicular cycle of the lizard *Tropidurus torquatus* (Squamata, Tropiduridae) in the Cerrado of central Brazil. *Amphibia-Reptilia* **22**: 217-233.
- Vitt, L.J. (2013): Walking the natural-history trail. *Herpetologica* **69**: 105-117.

- Vitt, L.J., Ávila-Pires, T.C.S., Zani, P.A. (1996): Observations on the ecology of the rare Amazonian lizard, *Enyalius leechii* (Polychrotidae). *Herpetol. Nat. Hist.* **4**: 77-82.
- Vitt, L.J., Price, H.J. (1982): Ecological and evolutionary determinants of relative clutch mass in lizards. *Herpetologica* **38**: 237-255.

## APPENDIX

**Appendix 1.** List of museums and voucher specimens of *Enyalius perditus* examined.

| Collection (Abbreviation)  | Voucher number  |
|--|---|
| Instituto Butantan, São Paulo, Coleção de Referência (IBSPCR)      | SÃO PAULO: São José do Barreiro (IBSPCR 407).   |
| Museu de Zoologia, Universidade Estadual de Campinas (ZUEC)        | SÃO PAULO: Ilhabela (ZUEC 2934, 2935, 2936, 2938, 2942), Ubatuba (ZUEC 1887).   |
| Museu de Zoologia João Moojen (MZUFV)                              | MINAS GERAIS: Lambari (MZUFV 633).  |
| Coleção Herpetológica Alfred Russel Wallace (CHARW)                | MINAS GERAIS: Lambari (CHARW 94, 155, 158, 160, 161, 194, 289, 293, 296, 321), Alfenas (CHARW 322), Boa Esperança (CHARW 317).  |
| Coleção Herpetológica da Universidade Federal de Ouro Preto (UFOP) | MINAS GERAIS: Itatiaia, Serra de Ouro Branco (UFOP 939s, 968s, 976s, 978s, 993s, 995s, 996s, 997s, 1042s, 1062s, 1077s, 1087s). |
| Museu de História Natural Capão da Imbuia (MHNCI)                  | PARANÁ: Telêmaco Borba (MHNCI 3128, 12956, 12966).  |