

## Reproductive biology of *Echinanthera cyanopleura* (Serpentes: Dipsadidae) in southern Brazil

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**ABSTRACT.** The reproductive cycles of snakes can be influenced by many factors, both biotic and abiotic, and information about these factors can contribute significantly to knowledge of the biology of many species. Here, we present data on the reproductive biology (body size, sexual dimorphism and female reproductive cycle) of the forest-dwelling colubrid *Echinanthera cyanopleura* (Cope, 1885), based on analyses of 128 specimens preserved in collections and originating from the states of Paraná, Santa Catarina, and Rio Grande do Sul in southern Brazil. The snout-vent length of females was significantly greater than in males. The tail length of mature females was greater than in males, although this difference was not significant. Vitellogenesis occurs from August to December and eggs were found in greater frequency from October through December. Juveniles were found in larger numbers beginning in February, indicating that recruitment occurs from January to April. The reproductive cycle of this species is seasonal, which is usual for oviparous colubrids of temperate areas of Brazil.

**KEY WORDS.** Reproduction; serpents; sexual dimorphism.

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The colubrid species of *Echinanthera* Cope, 1894 are distributed in South America. *Echinanthera cyanopleura* (Cope, 1885) occurs in southeastern and southern Brazil, from central-western Espírito Santo to southeastern Rio Grande do Sul, including the states of Minas Gerais, Rio de Janeiro, São Paulo, Paraná, and Santa Catarina (DI-BERNARDO 1992). This snake is middle-sized, diurnal and inhabits forests. Little is known about the reproductive biology of this species and the available studies have drawn on small samples (GIRAUDO 2001, MARQUES & SAZIMA 2004).

The sparse information about the reproductive biology available in the literature does not allow the reproductive cycle of this species to be determined. Here, we present data on the sexual dimorphism in body size, female reproductive cycle and recruitment of *E. cyanopleura* in southern Brazil.

### MATERIAL AND METHODS

This study was based on the dissection of 128 specimens of *E. cyanopleura* from southern Brazil, including the states of Paraná, Santa Catarina, and Rio Grande do Sul. The specimens are deposited in the following institutions: Universidade de Passo Fundo (CRUPF), Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCP), Museu de Ciências Naturais da Fundação Zoobotânica (MCN),

Instituto Butantan (IB), Universidade Federal de Santa Maria (ZUFMS), and Museu de História Natural Capão da Imbuia (MHNCI) (Appendix 1).

For each specimen, we recorded the following data: snout-vent length (SVL, mm), tail length (TL, mm), total length (TL, mm), sex (analysis of gonads), size and number of vitellogenic follicles or eggs (mm) and condition of efferent ducts.

The reproductive season, as defined to here, is the period of secondary vitellogenesis to the clutch, and fertility is defined with respect to the number of follicles in secondary vitellogenesis, eggs in the oviduct or number of eggs in the hatchlings.

Males with convoluted and opaque efferent ducts were considered mature (SHINE 1988). Females that had follicles in secondary vitellogenesis (diameter greater than 10 mm) or eggs were considered mature (SHINE 1988). The size of follicles was determined with the aid of a caliper (mm), to a precision of 0.01.

We excluded from the analysis the females that had juvenile-like follicles, but that had an extended oviduct. The degree of sexual size dimorphism (SSD) was calculated as  $1.0 - (\text{mean SVL of the larger sex} / \text{mean SVL of the smaller sex})$  (SHINE 1994).

For the analysis of sexual dimorphism in body size, we did not include specimens for which the SVL could not be measured or that had an amputated tail.

For the statistical analysis, the  $\chi^2$  ANOVA was used, at the 5% significance level. Statistical tests were conducted using BioEstat 5.0.

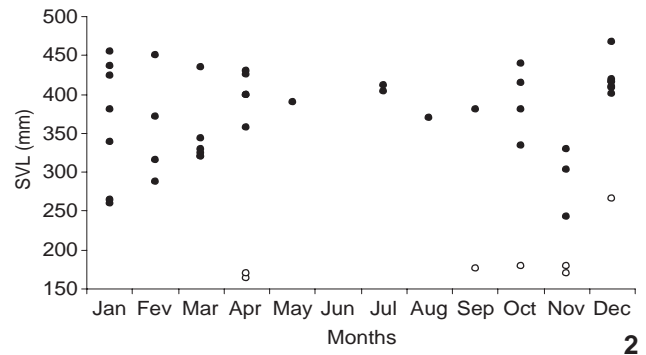
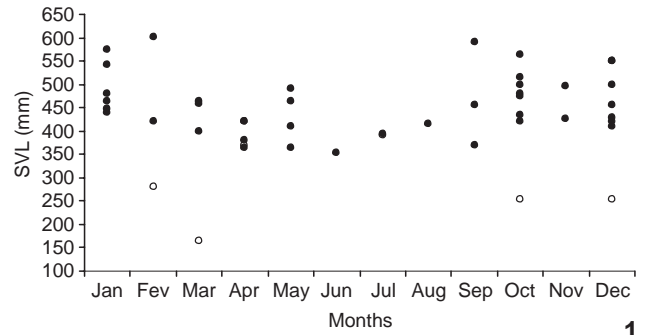
**RESULTS**

In mature females, the SVL ranged from 354 to 602 mm ( $\chi = 462$  mm, SD = 6.2, n = 42). Above 395 mm, all the females were mature. The TL ranged from 170 to 244 mm ( $\chi = 202$  mm, SD = 2.2, n = 29) (Fig. 1). In the mature males, SVL ranged from 243 to 467 mm ( $\chi = 376$  mm, SD = 5.7, n = 41). All males longer than 288 mm were mature. The TL ranged from 114 to 231 mm ( $\chi = 182$  mm, SD = 2.9, n = 35) (Fig. 2). The SVL of mature females was significantly greater than that of mature males ( $t = 6.6$ ,  $p < 0.001$ ,  $gl = 75$ ) and there was no significant difference between the TL of mature males and females ( $t = 0.14$ ,  $p = 0.88$ ,  $gl = 62$ ). Of all specimens analysed, 10.1% (n = 11) of the females and 4% (n = 2) of the males had mutilated tails. The degree of sexual size dimorphism was calculated as 0.21.

Females showed a seasonal reproductive cycle, with vitellogenesis occurring from August through December. Pregnant females with eggs in the oviduct were found most frequently from October through December. There was one record of a pregnant female in September and one in February (Fig. 3). A female collected on November 25<sup>th</sup>, 2002 (CRC = 425 mm) laid six eggs in the laboratory, one egg on December 5<sup>th</sup> and five eggs on December 6<sup>th</sup>; the eggs ranged in size from 24 mm to 32 mm ( $\chi = 27$  mm; SD = 3.4). All the eggs deteriorated (Tab. I).

Males and females were collected in higher numbers in the warmer months (October to April) ( $\chi^2 = 38.22$ , n = 71). Females were collected in higher numbers in October; more males than females were collected from November through April. Based on records of the period when females had eggs in the oviduct, the record of a clutch laid in captivity in December, and a higher frequency of juveniles beginning in February, it appears that the recruitment period occurs from January through April (Fig. 4).

The number of vitellogenic follicles in *E. cyanopleura* ranged from five to nine ( $\chi = 5.8$ , SD = 2.21, n = 4), and the number of eggs in the oviduct ranged from three to nine ( $\chi = 5.3$ , SD = 2.05, n = 11). The difference between the number of vitellogenic follicles and eggs found in the oviduct was not significant (Mann-Whitney test, U = 19.0, Z = 0.3917,  $p = 0.6953$ , n = 16), suggesting that the clutches can be regarded



Figures 1-2. Seasonal variation in snout-vent length in *E. cyanopleura* from southern Brazil. (1) Females: (●) mature (n = 41), (○) immature (n = 6); (2) Males: (●) mature (n = 41), (○) immature (n = 8).

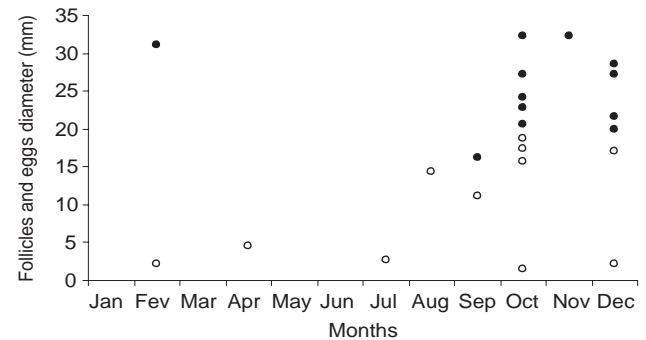


Figure 3. Seasonal variation in the size of ovarian follicles (○) (n = 11) or eggs (●) (n = 12) of *E. cyanopleura* from southern Brazil.

Table I. Seasonal occurrence of females in secondary vitellogenesis, pregnant females, and clutches of *Echinanthera cyanopleura* from southern Brazil.

	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Vitellogenesis			■	■	■	■	■					
Pregnant female				■	■	■	■		■			
Clutches							■					

as the number of vitellogenic follicles or eggs found in the oviduct. The clutch size and SVL of females showed a highly significant correlation (Pearson's linear correlation;  $t = 3.98$ ,  $r = 0.7292$ ,  $p = 0.0013$ ,  $n = 16$ ).

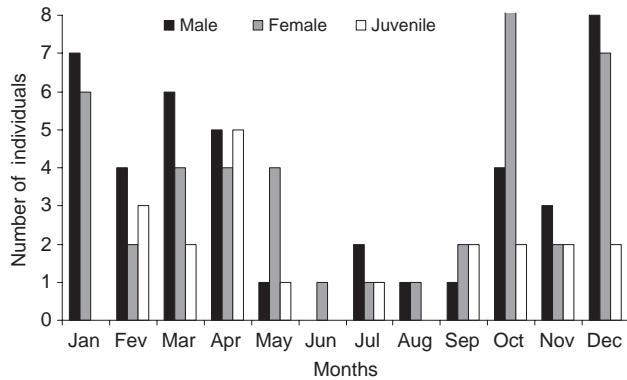


Figure 4. Seasonal abundance of males and females of *E. cyanopleura* from southern Brazil. Black bars represent mature males ( $n = 42$ ), grey bars represent mature females ( $n = 43$ ) and white bars represent immature juveniles ( $n = 20$ ).

## DISCUSSION

Females of *E. cyanopleura* are larger than males, as with other species of Dipsadidae such as *Erytrolamprus aesculapii* (Linnaeus, 1766) (MARQUES 1996), *Liophis poecilogyrus poecilogyrus* (Wied, 1825) (PINTO & FERNANDES 2004), *Helicops infrataeniatus* (Jan, 1865) (AGUIAR & DI-BERNARDO 2005) and *Philodryas agassizii* (Jan, 1863) (MARQUES *et al.* 2006). The larger SVL in females is the more common pattern in snakes, and may be related to the absence of combat behavior between males (SHINE 1994).

There is no record of combat between males in *E. cyanopleura* and therefore this finding corresponds to the expected pattern in relation to body size. A larger female body size can offer advantages, such as increasing fecundity by allowing more clutches and litters, thus providing greater reproductive fitness (RIVAS & BURGHARDT 2001). Increase in fecundity can be achieved by two basic strategies: 1) producing more eggs of smaller size; 2) producing fewer eggs of larger size.

The data presented here suggest that *E. cyanopleura* produces a small clutch (three to nine), similar to *E. undulata* (Wied, 1824) (three to eight eggs) (MARQUES & SAZIMA 2004) in the Estação Ecológica Juréia-Itatins, São Paulo. In oviparous snakes, the eggs represent the major reproductive investment by females, with eggs representing a major investment in clutch mass (SINERVO 1993). *E. cyanopleura* is a medium-sized terrestrial-cryptozoic species. The clutch mass is composed of elongated eggs, arranged in a single series and occupying a large part of the body of the female. This strategy may be associated with a lower mortality rate of nestlings, because larger eggs

mature more rapidly, which may compensate for the low fertility rate recorded for this species. One of the strategies adopted by snakes that lay fewer eggs per clutch is to invest in larger eggs (see PONTES & DI-BERNARDO 1998).

In southern South America, oviparous colubrids have markedly seasonal reproductive cycles (PONTES & DI-BERNARDO 1988), coinciding with warmer periods of the year. This pattern was observed for *E. cyanopleura* by MARQUES & SAZIMA (2004) in the Estação Ecológica Juréia-Itatins, São Paulo, and in the present study. The vitellogenesis of *E. cyanopleura* occurs from August to February. The record of a clutch in December is consistent with the pattern found for other oviparous colubrids, such as *Atractus reticulatus* (Boulenger, 1885), with vitellogenesis occurring from August through December (BALESTRIN & DI-BERNARDO 2005).

The pattern found for *E. cyanopleura* in relation to the size of the tail (there was no significant difference between males and females) does not correspond to the general pattern found in snakes, in which males have longer tails than females (KING 1989, SHINE 1993, BALESTRIN & DI-BERNARDO 2005, AGUIAR & DI-BERNARDO 2005). Body size is a major determinant of the type of prey that a species can exploit (CADLE & GREENE 1993) and the absence of dimorphism in the tail may be related to ecological factors, such as foraging strategy and defense mechanisms. There are records of amphibians in the diet of this species, both in temperate southern Brazil and in a tropical area (São Paulo). MARQUES & SAZIMA (2004) suggested that *E. cyanopleura* can use its long tail to search the substrate and disturb the amphibians living in the forest litter, which may help them to locate their prey. Some defensive displays are associated with the microhabitat used by this snake: tail and horizontal head displays were found mostly in terrestrial species (GREENE 1979). The tail may also be used for self-defense: specimens in herpetological collections show high levels of mutilation (33%,  $n = 12$ ) (MARQUES & SAZIMA 2004). In this study, we found that 12% ( $n = 13$ ) of the specimens had amputated tails, which corroborates the hypothesis that tails are used for self-defense.

Low temperatures may reduce the metabolic rate of snakes and consequently reduce their activity (LILLYWHITE 1987). Our data indicate that *E. cyanopleura* presents seasonal activity, because it is most active in warm periods of the year, from October to April, with recruitment from January to April.

A similar activity pattern was observed for *A. reticulatus*, with recruitment during the warm months of the year (BALESTRIN & DI-BERNARDO 2005). In the temperate region of Brazil, temperature is one of the main factors responsible for the low activity of snakes (DI-BERNARDO *et al.* 2007), whereas in tropical areas of Brazil, the main factor seems to be rainfall (MARQUES *et al.* 2000). Other factors that are not abiotic in nature may also play a role in the increased activity of *E. cyanopleura* in the warmer months of the year, such food availability. Anurans, the favored prey (MARQUES & SAZIMA 2004) are most abundant in spring and summer (BERNARDE & MACHADO 2001, GRANDO *et al.* 2004), and could be factor influencing the activity, patterns of *E. cyanopleura*.

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## Appendix 1. Examined material.

Brazil. Rio Grande do Sul: Bagé: MCN 2749; Cachoeira do Sul: MCP 11207; Cambará do Sul: MCP 14415; Caxias do Sul: IB 29025, 29026, MCP 12191; Don Pedro de Alcântara: MCP 7899; Estância Velha: MCN 7837; Gramado: MCP 13285, MCN 12939; Marau: CRUPF 1135; Canela: MCN 6446, 13140, 14012; Machadinho: MCN 14310; Morro Reuter: MCN 14684; Nova Petrópolis: MCP 12189; Passo Fundo: CRUPF 093, 583, 636, 654, 693, 715, 803, 695, 867, 804, 881, 862, 878, 890, 893, 902, 913, 919, 929, 930, 943, 944, 945, 954, 962, 963, 964, 965, 966, 970, 978, 986, 996, 1067, 1069, 1096, 1117, 1129, 1143, 1137, 1172, 1173, 1174, 1175 1185, 1188, 1189, 1195, 1197, 1244, 1447, 1448, 1458, 1465, 1490, 1523, 1684, 1686; Porto Alegre: MCN 1512; MCP 2468, 10990, 11794; Sertão: CRUPF 1684, 1686; Santa Maria: ZUFMS 2438, 2569; São Leopoldo: IB 5686, 6665, 7775, 8319,

10254; MCN 5901; Taquara: MCP 11389; Torres: MCN 2747; Santa Cruz do Sul: MCN 7740; São Francisco de Paula: MCN 4619, 6445, MCP 10996; Sapucaia: MCN 8853; Tapes: MCP 14489; Tenente Portela: MCN 7201; Veranópolis: IB 9886, Viamão: MCN 2688. Paraná: Biturama: IB 19491; Cutitiba: MHNCI 2233; Mallet: IB 23429, 49452; Mangueirinha: MHNCI 5314; Morretes: MHNCI 11618; Piraquara: MHNCI 7537; Rio Azul: IB 7086, 24645; Teixeira Soares: MHNCI 8947; União da Vitória: IB 25182; Santa Catarina: Blumenau: IB 40112, 40117; Campina Grande: MHNCI 9403; Campo Tenente: IB 6691; Corupá: IB 6011, 8264, 10333; Fazenda Rio Grande: MHNCI 11019; Joenvile: IB 24469; Mangueirinha: MHNCI 5313; Videira: IB 16615, 27888; Rio Antas: IB, 4515, 9902; Rio Azul: IB 7563; Reserva do Iguaçu: MHNCI 5117; São Mateus: MHNCI 797.

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