Reproductive biology of the swamp racer *Mastigodryas bifossatus* (Serpentes: Colubridae) in subtropical Brazil

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**ABSTRACT.** The swamp racer *Mastigodryas bifossatus* (Raddi, 1820) is a large snake of Colubrinae. It is widely distributed in open areas throughout South America. Dissection of 224 specimens of this species housed in herpetological collections of the southern Brazilian states of Rio Grande do Sul, Santa Catarina and Paraná provided information on its sexual dimorphism, reproductive cycle and fecundity in subtropical Brazil. Adult specimens of *M. bifossatus* average approximately 1190 mm in snout-vent length and females are larger than males. The reproductive cycle of females is seasonal, with secondary vitellogenesis occurring from July to December. However, examination of male gonads did not reveal signs of reproductive seasonality in this sex. Egg laying was recorded from November to January. The estimated recruitment period extends from February to April. The mean number of individuals per clutch is 15, and there is a positive correlation between female length and clutch size.

**KEY WORDS.** Fecundity; neotropics; reproduction; reproductive cycle; sexual dimorphism.

*Mastigodryas* Amaral, 1935 belongs to Colubrinae and contains large-sized, generally robust species, active during the day, and can display aggressive behavior (CEI 1993). The genus contains 11 species distributed from Mexico to southern Brazil, Bolivia, Paraguay and northern Argentina (PETERS & OREJAS-MIRANDA 1970, LEMA 1994, GIRAUDE 2001).

*Mastigodryas bifossatus* (Raddi, 1820) occurs from Venezuela and Colombia to northern Argentina (PETERS & OREJAS-MIRANDA 1970). Along its wide distribution range throughout the Neotropical region, five subspecies were recognized but subsequently invalidated, and are currently considered morphotypes (see OUTERAL & LEMA 2003). In Brazil, *M. bifossatus* occurs predominantly in open areas of the Cerrado, Pantanal and Pampa biomes, and also occurs, in lower abundance, in the Amazonian and Atlantic forests (STRESSMANN & SAZIMA 1993, LEMA 2002, ARGÔLO 2004, MARQUES et al. 2004, MARQUES & MURIEL 2007).

The good representation of *M. bifossatus* in Brazilian herpetological collections has allowed studies on its ecology. Aspects of the natural history of *M. bifossatus* in southeastern South America were characterized by MARQUES & MURIEL (2007). LEITE et al. (2007) provided information on its diet and habitat use in southern Brazil. Previous reports consist of occasional observations on the period of activity and spawning and food preferences, with no characterization of its reproductive cycle (AMARAL 1978, STRESSMANN & SAZIMA 1993, MARQUES et al. 2004). In this study we present information on sexual dimorphism, maturity, reproductive season, spawning period, recruitment and fecundity of *M. bifossatus* (morphotype *M. b. bifossatus*) from subtropical domains of Brazil.

**MATERIAL AND METHODS**

**Study area**

Most of the subtropical region in Brazil corresponds to the states of Paraná, Santa Catarina and Rio Grande do Sul. The seasons are well defined, and precipitation is more or less evenly distributed throughout the year (KOEPPE 1948). The vegetation includes several physiognomies such as savannas, steppes, dense and mixed rainy forests, and seasonal deciduous and semideciduous forests, which are distributed basically according to altitude and latitude (LEITE 2002), and also influenced by temperature and precipitation patterns (RODERJAN et al. 2002). In the southern Brazilian highlands, mixed rainforest is prevalent, where *Araucaria angustifolia* (Bertol.) Kuntze is the predominant species (RODERJAN et al. 2002). Dense rainforest remnants of the Atlantic Forest predominates in the Serra do Mar range along the three states (RODERJAN et al. 2002). The southern portion of Rio Grande do Sul state includes part of the Pampa biome (MARCHIORI 2002, WAECHTER 2002), whereas in northern and northeastern Paraná, small portions of Cerrado (savanna) occur (RODERJAN et al. 2002).

**Methods**

Data were obtained by dissection of 224 specimens of *M. bifossatus* preserved in the herpetological collections of the
Universidade Federal de Santa Maria (ZUFSM, n = 108); the Museu de Ciências e Tecnologia of the Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP, n = 48); the Instituto Butantan, São Paulo (IB, n = 34); the Museu de História Natural do Capão da Imbuia, Curitiba (MHNCI, n = 19) and the Museu de Ciências Naturais of the Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre (MCN, n = 15). For each specimen we recorded the snout-vent length (SVL, in mm), tail length (TL, in mm), head length (HL, in mm), sex (gonad analysis), number and length (in mm) of vitellogenic follicles or oviductal eggs, length and width (both in mm) of testicles, and the state of the deferent ducts.

Specimens examined

The reproductive season was defined as the period from secondary vitellogenesis to the egg laying. Data on newborns came from captive laying. Captive females were taken from the field and maintained individually at natural temperature in sand-filled glass terraria (100 x 40 x 40 cm), with water supply, and were fed on mice. Fecundity was estimated by the number of follicles in secondary vitellogenesis, oviductal eggs and number of eggs per clutch.

RESULTS

Sexual dimorphism

SVL of newborns ranged from 270 to 315 mm (mean = 287, sd = 16, n = 7) and TL from 97 to 117 mm (mean = 106, sd = 7.5, n = 7). Mature male SVL ranged from 830 to 1480 mm (mean = 1113, sd = 145, n = 101), and TL from 295 to 520 mm (mean = 408, sd = 46, n = 81). Mature female SVL ranged from 930 to 1560 mm (mean = 1190, sd = 139, n = 87) and TL from 295 to 484 mm (mean = 395, sd = 39, n = 63). Mature females are larger in size than mature males (Mann-Whitney, U = 3193.5, Z = 3.2, p = 0.001), and the degree of sexual size dimorphism in body size is 0.07. Adult males and females did not differ in head length (ANOVA, F = 0.02, p = 0.87, n = 177). However, adult males had a larger relative TL (ANOVA, F = 54.55, p < 0.0001, n = 157) than females (Fig. 1). Among the analyzed specimens 22.76% (n = 51) had damaged tails (20% in males, n = 20, and 27% in females, n = 31).
Reproductive season, spawning period and birth of the nestlings

*Mastigodryas bifossatus* shows secondary vitellogenesis from July to December, while oviductal eggs from October to December (Fig. 4), indicating a seasonal reproductive cycle for females. Egg-laying was recorded from November to January (n = 3) in captivity. A female collected on May 2nd, 2004 and kept in captivity laid 14 eggs on January 13, 2005, of which only 3 eggs completed their development. The mean SVL, TL and weight of the newborns were, respectively, 305 mm (range = 295-315 mm, sd = 10), 104.6 mm (range = 102-109, sd = 3.78) and 8.6 g (range = 7.5-9.7 g, sd = 1.1). A female collected on November 9, 1997 laid 22 eggs, of which four newborn erupted in February 1998. The mean SVL and TL of the newborns were, respectively, 274.25 mm (range = 270-278 mm, sd = 3.3; n = 4) and 107 mm (range = 97-117, sd = 9.9; n = 4). Another female collected on November 10, 2005 laid 10 eggs on January 09, 2006, but no nestlings erupted from the clutch.

Five females had, concomitantly, oviductal eggs and follicles in secondary vitellogenesis. One female analyzed immediately after laying also had vitellogenic follicles.

There was no difference in relative testicular volume among seasons (ANCOVA, F = 2.10, p = 0.10, n = 84).

Sexual maturity

The smallest mature male was 830 mm in SVL. The SVL of the smallest mature female was 930 mm. The seasonal distribution of the snout-vent length of *M. bifossatus* indicated that the body size of individuals almost doubles during the first year, and males and females attain sexual maturity between the ages of 18 and 24 months respectively (Figs 2 and 3).

Fecundity

The number of vitellogenic follicles in *M. bifossatus* ranged from 3 to 31 (mean = 14.11, sd = 7.84, n = 36), the number of oviductal eggs ranged from 8 to 22 (mean = 13, sd = 5.5, n = 7) and the number of eggs per clutch ranged from 10 to 22 (mean = 15, sd = 6, n = 3). There was no significant difference between the mean number of follicles in secondary vitellogenesis and the mean number of oviductal or laid eggs (Mann-Whitney, U = 123, Z = 0.209, p > 0.05, n = 44). Clutch size and female SVL showed a significant positive correlation (Pearson, r = 0.34, p < 0.05, n = 45) (Fig. 5).
DISCUSSION

Sexual dimorphism

Differences in body size between males and females are considered evolutionary adaptations that increase reproductive success in snakes (Shine 1978). These differences can be interpreted to other observed patterns such as male-male combat, the relationship between female body size and clutch, reproductive frequency and geographic distribution of individuals (Shine 1978, 1994a). In most cases, female colubrids attain larger SVL than conspecific males (Fitch 1981). The small difference in size between males and females of M. bifossatus (ISD = 0.07) can be related to increased fecundity in females (Shine 1993, 1994a).

Male-male ritual combats in M. bifossatus have not been reported, and there are no known cases in the genus. In Colubridae, this behavior is reported mainly for Colubrinae (Shine 1994a), for instance Chironius carinatus (Linnaeus, 1758) (Starace 1998) in the Amazon and Chironius bicarinatus (Wied, 1820) (Almeida-Santos & Marques 2002) in southeastern Brazil.

Considering that the presence of this behavior influences the degree of sexual dimorphism (Shine 1978, 1994a), we cannot discard the hypothesis that M. bifossatus has male-male combat, because colubrids with a similar degree of sexual dimorphism, such as the colubrine Masticophis lateralis (Hallowell, 1853), show this type of behavior (see Shine 1994a). Moreover, analyses of specimens of M. bifossatus from southeastern Brazil indicated equivalent body sizes for males and females (Marques & Muriel 2007). However, we highlight that field behavioral observations are needed to evaluate the existence of male-male combat in this species.

The lack of sexual dimorphism in head size of snakes was related to intersexual diet similarities (Shine & Crew 1988). This hypothesis was corroborated by Leite et al. (2007), who did not observe any difference in prey size ingested by male and female M. bifossatus in subtropical Brazil.

Males of M. bifossatus have a proportionally larger relative tail length than females. This pattern is very common in snakes (Shine 1993), and is due mainly to the hemipenial accommodation (King 1989). Tail length also influences male reproductive success, acting as a sexual selection factor (Shine et al. 1999). Masticophis bifossatus has a quite long tail for a species with preferentially terrestrial habits. In this species, this character can be related to the capacity for autotomy, a poorly known tactic employed as a defensive mechanism by both sexes (Arnold 1988, Prudente et al. 2007).

The data obtained in this study confirm the high incidence of tail breakage in M. bifossatus (22.76%), as previously recorded for specimens from various localities in Brazil (46.7%, Ferreira & Outiberal 1998). Other colubrids, such as Coniophanes fissidens (Günther, 1858) and Dendrophidion dendrophis (Schlegel, 1837), also show this type of damage (Zug et al. 1979, Prudente et al. 2007). Tail breakage can act as an anti-predator and escape mechanism (Zug et al. 1979, Prudente et al. 2007), and is used by many lizard and some snake species (Arnold 1988). According to White et al. (1982), the incidence of this type of damage increases in larger individuals.

Sexual maturity

Differences in growth rates among species or even within a species are related to the climate conditions of each geographical locality, especially the length of the growing season (Luisselli et al. 1997). The same may occur with M. bifossatus, because individuals that were born at the beginning of the recruitment period (February) enjoy a longer growth period before winter than those that hatch at its end (May). This allows males of small-sized species such as Atractus reticulatus (Boulenger, 1885) and Lystrophis dorbignyi (Duméril, Bibron & Duméril, 1854) attain sexual maturity at smaller sizes than females, soon in their first reproductive season (Shine 1980, Marques & Puerto 1998, Balestrin & De-Bernardo 2005). However, in spite of the duplication of the body size in male M. bifossatus in the first year of life (Marques & Muriel 2007, this study), sexual maturity is probably reached only in the second reproductive season. In females, the age of sexual maturity may be delayed in relation to conspecific males, because of the high energetic costs of reproduction (Shine 1978). Females must reach larger body sizes in order to produce larger egg masses and, consequently, more nestlings (Luisselli et al. 1996).

Reproductive season, laying period and birth of the nestlings

Females of M. bifossatus showed reproductive seasonality, with secondary vitellogenesis lasting approximately five months in southern Brazil. A similar pattern was previously observed for several snake species in subtropical regions (Duellman 1978, Vitt 1983, Vitt & Vangilder 1983). Nevertheless, in the lower latitudes of southeastern South America, Marques & Muriel (2007) classified the reproductive cycle of M. bifossatus as continuous, with ovarian follicles in vitellogenesis found virtually during all months of the year. Additionally, these authors indicated pronounced reproductive season-
ality for this species, with recruitment peaking at the end of the rainy season. Our results suggest that *M. bifossatus* shows a more-pronounced reproductive seasonality in the subtropical region, with absence of vitellogenesis during the coldest months and an estimated recruitment period that extends from February to April.

Phylogenetic factors play an important role in determining reproductive seasonality in snakes (Seigel & Ford 1987). However, the seasonal pattern is probably imposed by the pronounced temperature variations in the region studied, which restricts the occurrence of reproduction to the warmest months of the year (Pontes & Di-Bernardo 1988, Di-Bernardo et al. 2007), as in snakes in temperate climates (Kofron 1983). Studies in southeastern Brazil have also indicated reproductive seasonality in species of Colubrinae, such as *C. bicarinatus* (Marques & Sazima 2004), *Spilotes pullatus* (Linnaeus, 1758) (Marques & Sazima 2004, Hauzmann et al. 2005) and *Tantilla melanochephalus* (Linnaeus, 1758) (Marques & Puorto 1998).

Factors such as temperature and reproductive condition in females can influence testicular development in male snakes (Alridge 1975, Bull et al. 1997). In *M. bifossatus*, the testicular relative volume did not show significant changes during the year. This indicates an absence of reproductive seasonality, because the increase in testicular volume is positively related to the degree of sexual hormone expression (Merila & Sheldon 1999). One of the factors responsible for reproductive non-seasonality in subtropical male snakes seems to be the absence of a pronounced dry season (Saint-Girons 1982). In addition to this factor, we believe that the reproductive asynchrony between male and female *M. bifossatus* reflects the lower energetic cost for production of the male gametes (see Olsson et al. 1997 for discussion).

Fecundity

The available data indicate that *M. bifossatus* can produce from 8 to 18 eggs (Amaral 1978, Leitão de Araújo 1978). However, in this study we found specimens containing up to 22 eggs. The mean number of 15 eggs found for the species in the present study is relatively low considering the large body size of this species, but it is similar to that found for the same species in southeastern South America (see Marques & Muriel 2007). Smaller-sized Neotropical colubrids, such as *Philodryas patagoniensis* (Girard, 1858) and the viviparous *Tomodon dorsatus* Duméril, Bibron & Duméril, 1854, show fecundity similar to *M. bifossatus* (Fowler et al. 1998, Bizerra et al. 2005). This can be explained by the larger size of *M. bifossatus* newborns as compared to those of other Neotropical oviparous snakes (Pontes & Di-Bernardo 1988).

The positive correlation between snake size and number of eggs in *M. bifossatus* reinforces a broadly documented intraspecific pattern (Seigel & Ford 1987, Shine 1994b) recorded in specimens of this species from lower latitudes in Brazil (Marques & Muriel 2007) and in various Neotropical colubrid species, such as *Philodryas olfersii* (Lichtenstein, 1823), *P. nattereri* (Steindachner, 1870) (Vitt 1980), *Sibon sanniola* (Cope, 1867) (Kofron 1983) and *T. melanochephalus* (Marques & Puorto 1998).

As suggested in a study on *M. bifossatus* in southeastern South America (Marques & Muriel 2007), we believe that it is capable of multiple egg layings. This hypothesis is based on the large number of follicles in secondary vitellogenesis and the simultaneous presence of oviductal eggs in some females, although the conversion of follicles in eggs in the same reproductive season is not guaranteed (Seigel & Ford 1987). Multiple egg layings were recorded in *Tropidonophis mairii* (Gray, 1841) in nature (Brown & Shine 2002). There are records of multiple egg layings in *Micrurus corallinus* (Merrem, 1820) and *Simophis rhinostoma* (Schlegel, 1837) in captivity (Marques 1996, Jordão & Bizerra 1996), although these data can distort reproductive patterns in snakes, mainly because of the increased food availability in captivity (Seigel & Ford 1987).

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