Reproductive biology of the southern Brazilian pitviper *Bothrops neuwiedi pubescens* (Serpentes, Viperidae)

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Abstract. Dissection of 286 specimens of the *Bothrops neuwiedi pubescens*, combined with data on captive individuals, provided information on the reproductive biology of this viperid snake from southern Brazil. Females attained larger body sizes than males, and reproduction was seasonal with mating taking place in autumn when males were more frequently encountered. Vitellogenesis occurred from summer to spring (January-September), sperm storage during autumn and winter (May-September), ovulation and fertilization in early spring (September), embryonic development during middle spring and summer (October-March), and parturition in the summer (January-March). Embryonic development was estimated to last from three to five months, a shorter time than was previously reported. The number of offspring of *Bothrops neuwiedi pubescens* born in one litter can vary from four to 25 ($\bar{x} = 11$). Fecundity is correlated with maternal body size. Neonates measure 17-25 cm SVL. Inferred growth rate of juveniles was low (10 mm/month in first year), with males attaining sexual maturity at about 16 months, but maturity is delayed in females for at least two additional years.

Introduction

The pitvipers of the genus *Bothrops* are widely distributed in Central and South America (Campbell and Lamar, 1989). Reproductive data are available for only a few of the more than 20 species occurring in Brazil, such as *Bothrops jararaca* (Sazima, 1992; Janeiro-Cinquini et al., 1993; Almeida-Santos and Orsi, 2002), *B. moojeni* (Leloup, 1975), *B. atrox* (Hoge and Federsoni Jr., 1976) and *B. neuwiedi* (Alves et al., 1998, 2000; Valdujo et al., 2002). *Bothrops neuwiedi pubescens* inhabits deciduous forests of southern Brazil and Uruguay (Almeida, 1999). This species is a conspicuous component of the southern Brazilian snake fauna (Almeida, 1999; Cechin, 1999), and is responsible for many human snakebites in the region (Santos-Costa, 1999). Apart from scattered and anecdotal

reports (Melgarejo, 1978), scant information has been published regarding the reproductive ecology of this species. We provide data on body size, reproductive timing, clutch size, neonate size, and infer about the growth and age at maturity of this taxon.

Materials and methods

The present study is based on the dissection of 286 specimens of *Bothrops neuwiedi pubescens* from collections of the Museu de Ciências da Pontifícia Universidade Católica do Rio Grande do Sul (MCP), Universidade Federal de Santa Maria (ZUFSM) in Rio Grande do Sul, and of the Instituto Butantan (IB) in São Paulo. The specimens examined originated from Rio Grande do Sul state (27 to 32°S, 50 to 56°W), southern Brazil. This area is covered mainly by pampas grassland, deciduous forests, disturbed and cultivated areas (Lindman and Ferri, 1974; Vieira, 1984). The climate of the area is highly seasonal, with higher temperatures from spring to the onset of autumn and lower temperatures in the winter and most of the autumn. Rainfall is well distributed over most of the year (1600 mm) (Vieira, 1984; Melhem-Adas, 1996) (fig. 1).

The following data were taken from each specimen: (1) snout-vent length (SVL); (2) sex; (3) reproductive maturity (females were considered mature if they had embryos in oviducts or vitellogenic follicles; males were considered mature if they had enlarged testes or opaque deferent ducts indicating the presence of sperm, see Shine, 1980); (4) diameter of the largest ovarian follicle and the number and length (SVL) of embryos. Degree of sexual size dimorphism (SSD) was 1 — (mean adult SLV of the larger sex/mean adult SLV of the smaller sex) (see Shine, 1994). We inferred growth rates from seasonal distributions of body sizes, a method that relies on identification of age classes based on body sizes (Shine, 1978a). Data on captive snakes were obtained from registers of MCP. Seasonal activity

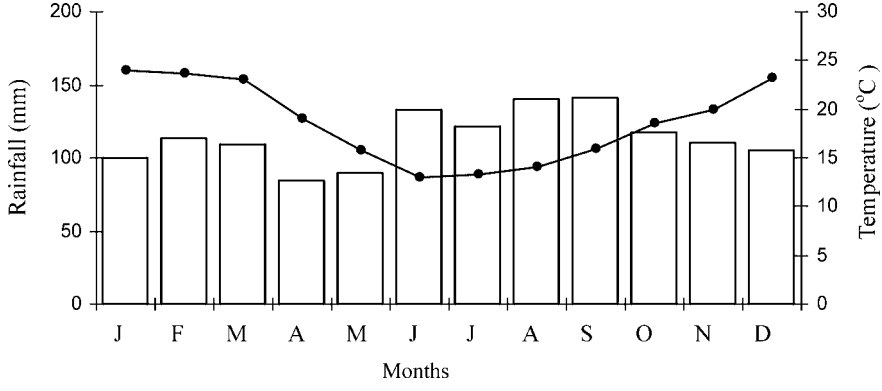

Figure 1. Annual distribution of mean monthly (°C) and rainfall (mm) in Rio Grande do Sul State from 1961 to 1990. Lines represent mean monthly temperature and bars denote rainfall. Data from Instituto Nacional de Metereologia, Brazil.

is based on collection dates of MCP, ZUFSM and IB specimens (see similar methods in Marques et al., 2001).

Results

Body size. *Bothrops neuwiedi pubescens* is a medium sized snake with adult SVL averaging 64.6 cm ($s = 14.2$, $n = 188$). Mature females averaged 76.2 cm SVL ($s = 10.9$, $n = 84$, range 62-111.5 cm), and mature males averaged 54.9 cm SVL ($s = 8.6$, $n = 104$, range 38-83). The difference in body size between sexes was highly significant ($t = 14.9$, df = 186, $P < 0.001$). The degree of sexual sex dimorphism (SSD) was 0.39.

Reproductive cycles. Enlarged follicles were present throughout the year (fig. 2). Ovulation occurred in early spring, and oviductal embryos were found from October to March (fig. 2). From October to December the embryos were in an early stage of development, and from January to March were fully-developed (fig. 2). Thus, we calculated that the gestation period extended from three to five months. The data from 11 captive gravid females are in agreement with those from dissected specimens, since parturition was recorded in January ($n = 3$), February ($n = 3$) and March ($n = 5$). One captive snake collected in April of 1996 and maintained alone gave birth on 16 January of 1998 (data from MCP), providing clear evidence of sperm retention.

Mating. Courtship of *B. n. pubescens* was observed in the field on day March 1997. The female (ZUFSM 1293) measured 850 mm SVL and the male (ZUFSM 1294) measured 595 mm SVL. Both individuals were mature, but the female had no vitellogenic follicles. Courtship and mating in captivity was recorded in July and August (G. Pontes, pers. com.).

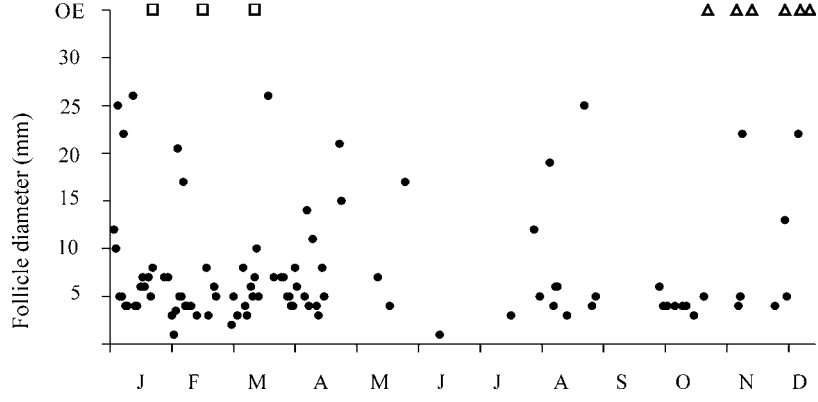

Figure 2. Seasonal variation in diameters of the largest ovarian follicles in adult female *Bothrops neuwiedi pubescens*. OE = oviductal embryos. Triangles represent early embryos (SVL < 4 cm) and squares represent fully-development embryos (SVL > 12 cm).

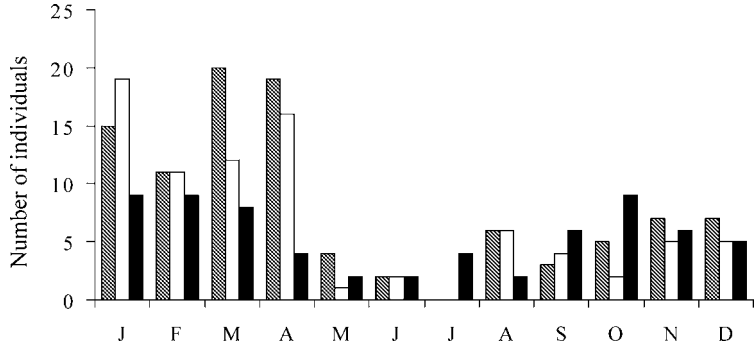

Figure 3. Seasonal abundance of male and females *Bothrops neuwiedi pubescens*, as show by collection data for museum specimens. Shaded bars represent adult males ($n = 99$), open bars are mature females ($n = 83$), and black bars are immatures ($n = 66$).

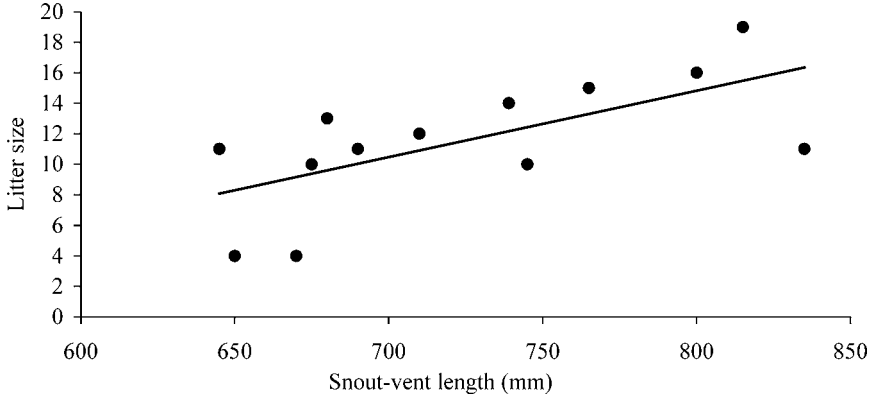

Figure 4. Relationship between female snout-vent length and litter size in *Bothrops neuwiedi pubescens*, from southern Brazil ($F_{1,11} = 25.3$, $r^2 = 0.669$, $P < 0.001$).

Seasonal activity. Adult males and females were collected year round, except for July (fig. 3). Both males and females were more often collected during the summer and onset of autumn (from January to April) ($\chi^2 = 105.09, n = 182$; df = 11; $P < 0.01$). The number of males collected outnumbered females in March and April (fig. 3). The frequency of juveniles was higher than expected from January to March and September to November, and lower than expected from April to August and in December ($\chi^2 = 16.02, n = 66$; df = 11; $P < 0.14$; fig. 3).

Litter size. The litter size averaged 11 ($s = 6.2$, range 4-25, $n = 13$) and was positively correlated with female body size (fig. 4). Neonates born in captivity averaged 21 cm SVL ($s = 2.1$ cm, range 17-25 cm, $n = 35$) and individual body mass averaged 10.12 g ($s = 4.65$ g, range 3.87-16.80 g, $n = 6$).

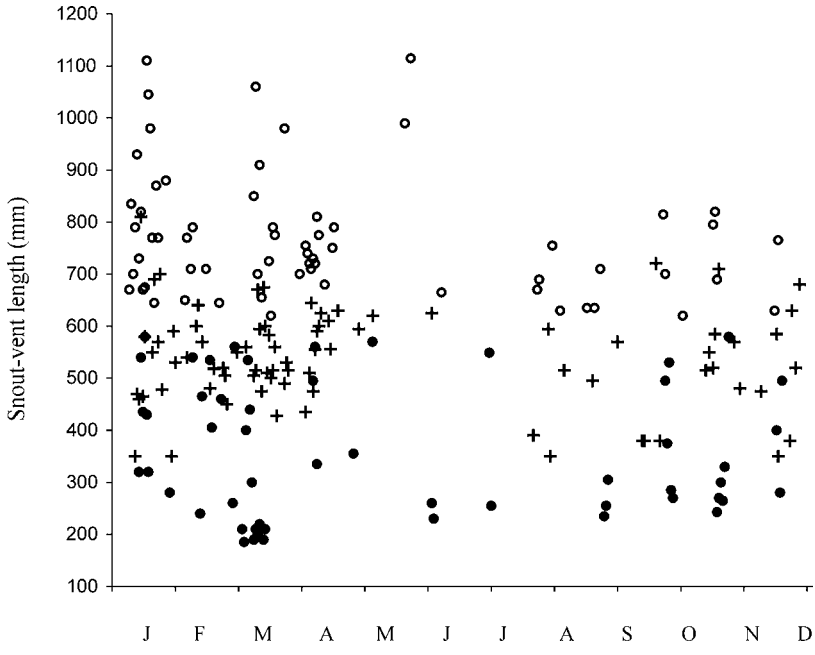

Figure 5. Seasonal distribution of body size of *Bothrops neuwiedi pubescens*, received in the Pontificia Universidade Católica from Rio Grande do Sul and Universidade Federal de Santa Maria from 1990 to 1997. Solid dots show juveniles, circles show mature females, crosses show mature males.

Growth rates. Seasonal distributions of SVL suggest period of recruitment and growth patterns of juvenile *B. n. pubescens* (fig. 5). Newborn snakes (SVL ~ 20 cm) appeared to occur in late summer and probably grew to a length of about 30 cm SVL by the end of the calendar year of their birth. Apparently, males attain maturity at an age of approximately 16 months, whereas maturity in females is delayed for at least two additional years.

Discussion

Females attain a much larger body size than males in *Bothrops neuwiedi pubescens*, similar to other species of the genus (Solórzano and Cerdas, 1989; Sazima, 1992; Sazima and Manzani, 1998; Nogueira, 2001). This is the most common condition among snakes and is generally correlated with absence of male-male combat behavior (Shine 1978b; Shine, 1994). Combat behavior in the genus *Bothrops* may be restricted to the *B. atrox* group, which includes *B. atrox*, *B. leucurus* and *B. moojeni* (see Almeida-Santos and Salomão, 2002), since our experience throughout many years at the Instituto Butantan (where thousands of specimens of *Bothrops,* including *B. neuwiedi* have been housed) male-male combat was only observed in three species of the *B. atrox* group. Moreover, *B. n. pubescens* have higher sexual dimorphism for size (SSD = 0.39) than members of

the *B. atrox* group in which male-male combat had been reported (0.15 for *B. atrox* and 0.10 for *B. moojeni*; Shine, 1994, and data obtained from Nogueira, 2001), suggesting that male-male combat behavior is absent in *B. n. pubescens.*

Reproductive cycles of this species seem to be seasonal and associated with warm periods of the year. Field data confirm full mating with a peak of male activity in March and April indicating mate-searching (Duvall et al., 1992). Embryonic development occurs in the spring and/or the summer when higher and more constant body temperatures are easier to maintain. High temperatures increase the rate of embryonic development and may reduce the incidence of anomalies (Vinegar, 1974). Recruitment of juveniles during warmer months is apparently widespread in the genus *Bothrops* (Sazima, 1992; Martins and Oliveira, 1999; Almeida-Santos and Salomão, 2002; Marques and Sazima, 2003). Most births occur when prey availability for juveniles is apparently high, since most frogs and lizards are abundant during the warm season in southern Brazil (Almeida, 1999; Cechin, 1999). Environmental factors such as humidity and temperature may be the main reasons for the presence of both young snakes and prey in the warmer months. Length of gestation in *B. neuwiedi* was estimated to be from 205 to 320 days in captivity (Alves et al., 1998), whereas we calculated it to be between 90 and 150 days. Studies of captive animals have often overestimated gestation length (e.g., Peñaranda, 1995; Cruz et al., 1989) because of sperm storage. Similar to data obtained here for one captive female, Alves et al. (1998) reported parturition to be 625 days after mating in another captive female of *B. n. pubescens.* These females apparently stored sperm for approximately sixteen months when their body mass started to increase, indicating the beginning of secondary vitellogenesis (Almeida-Santos and Salomão, 2002 — see Saint-Girons, 1975, 1982; Schuett, 1982, 1992; Almeida-Santos and Salomão, 1997; 2002 for discussion about storage of sperm). Mating in *B. n. pubescens* occurs in early autumn, with females storing sperm until spring when fertilization can occurs. *Bothrops neuwiedi* shows the presence of uterine muscular twisting (UMT) in non-vitellogenic and vitellogenic stages (Almeida-Santos and Salomão, 2002). The female observed in courtship in the field did not have vitellogenic follicles and probably did not give birth the following year. The data from animals in captivity confirm that females of species from the genus *Bothrops* have the ability to store sperm for more than one season by means of uterine muscular twisting (UMT) (see Almeida-Santos and Salomão, 2002).

The seasonal activity pattern recorded in *B. n. pubescens* seems to be related to the climatic variation in the region, with few active individuals during colder months. The few specimens collected during the winter were found on relatively warmer days (Almeida, 1999). In some temperate species, peaks of activity in spring and late autumn are associated to migration among different hibernation and foraging sites (Gibbons and Semlitsch, 1987). This does not seem to be the case in *B. n. pubescens*, as this species presents a unimodal seasonal activity pattern (with a peak from January to April). As reported for other species (Secor, 1994), it is possible that pregnant females of *B. n. pubescens* become more sedentary and probably inhabit secretive microhabitats, thus diminishing encounter

probability. A small number of pregnant females in the total sample could also be due to this sedentary behavior.

Encounter rate of males was higher in March and April, which could be explained by an increase in activity related to the mating period (cf. Secor, 1994). In juveniles, different selective pressures influence activity. Possibly, much of the time is devoted to foraging activities in order to assimilate energy for growth, and thus, activities may be less seasonal (Whiting et al., 1996).

The small body size is characteristic of the *B. neuwiedi* group and may be responsible for low size of neonates and low fecundity presented for *B. n. pubescens*, as well as *B. n. pauloensis* (Valdujo et al., 2002; see data for other *Bothrops* species in Leloup, 1975; Solórzano and Cerdas, 1989; Sazima, 1992). The inferred growth rate from seasonal distribution of juvenile *B. n. pubescens* is approximately 10 mm SVL/month. This value is within the range record for juvenile and adult of *B. jararaca* studied in the field (5-18 mm/month, see Sazima, 1992). The growth rate in *B. n. pubescens* is relatively low, similar to that verified for other viperids, which is probably due to the stout body of this group of snakes (see Shine, 1980). The long delay in sexual maturation recorded here is typical of other neotropical pitvipers (Sazima, 1992; MacCoy and Censky, 1992) and can be related to high survivorship of this group of venomous snakes (see Shine, 1980; Williams, 1996).

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