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Reproductive Biology of *Bothrops atrox* (Serpentes, Viperidae, Crotalinae) from the Brazilian Amazon

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Abstract: Reproductive seasonality is common among snakes, with mating, pregnancy, and birth or oviposition occurring only during few months of the year and modulated mainly by two environmental phenomena—temperature and rainfall. Species of *Bothrops* inhabit regions with varied climatic conditions, including those that are tropical, subtropical, equatorial, and semiarid. *Bothrops atrox* is an endemic and widespread species of the Amazon rain forest. Such habitat is characterized by an equatorial climate with little variability in temperature and humidity, but marked seasonality in intense precipitation. We investigated several aspects of the reproductive biology of *B. atrox*, including the reproductive cycle, the sexual segment of the kidney (SSK), sperm storage, and the possible influence of temperature and rainfall on pregnancy duration, birth, and spermatogenesis. Specifically, we examined museum specimens to describe sexual dimorphism, litter size, and male and female urogenital cycles through macroscopic and histological analyses. Females of *B. atrox* exhibited follicular recrudescence mainly from January to April (part of the wet season). Pregnant females were found throughout the year (except May), but births were found to occur mainly at the end of the dry season (August–October). Males exhibited SSK hypertrophy and sperm production in the wet season (November–April). Females were found to be larger than males, but males of *B. atrox*—as with other members of the *B. atrox* species complex—obtain relatively larger body sizes when compared with other species of *Bothrops*, which might be an adaptive response to male–male combat in this species. Together, our results show that *B. atrox* from Amazonia has a unique reproductive strategy (for each sex) among pit vipers, indicating greater reproductive plasticity when compared with its congeners.

Key words: Amazonian Lanceheads; Reproductive cycles; Sexual segment of the kidney; Sexual size dimorphism; Spermatogenesis; Vitellogenesis

Biotic and abiotic factors influence several organismal traits, including the timing and frequency of many reproductive events such as mating, vitellogenesis, pregnancy, and birth or oviposition, as well as spermatogenesis (Fitch 1982; Aldridge and Duvall 2002; Brown and Shine 2006; Krohmer and Lutterschmidt 2011). Temperature and rainfall are recognized as the main abiotic factors influencing reproductive cycles and maintenance of the hormonal peaks in snakes (Aldridge 1975; Aldridge and Duvall 2002; Lutterschmidt and Mason 2009).

Brazilian biomes differ widely in relation to thermal amplitude and rainfall. For example, high levels of annual rainfall (up to 3000 mm) and a humid climate throughout the year contribute to differences between the Amazon and both the Caatinga (annual precipitation 424–978 mm) and the Cerrado (annual precipitation ∼1500 mm; Nogueira et al. 2003; Nobre et al. 2009; Barros et al. 2014a). The climate of the Amazon is characterized by only slight variations in temperature throughout the year, but with rainfall seasonality, with a relatively rapid transition from the wet season to the dry season (Nobre et al. 2009).

Pit vipers of tropical (e.g., *Bothrops* and *Crotalus*) and temperate (e.g., *Agkistrodon*, *Crotalus*, and *Sistrurus*) zones show seasonal reproductive cycles, wherein mating can be temporally dissociated from fertilization and spermatogenesis (Aldridge and Duvall 2002; Almeida-Santos and Salomão 2002; Barros et al. 2012). In these genera, reproductive events—such as vitellogenesis and spermatogenesis initiation, hypertrophy of the sexual segment of the kidney (SSK), pregnancy, and birth—occur in warm seasons (Aldridge and Duvall 2002; Almeida-Santos and Salomão 2002; Siegel et al. 2009; Barros et al. 2012). The female reproductive cycle is highly conserved among species of *Bothrops*. Mating and sperm storage occur in autumn and winter (March–June [dry season] until September [storage]), whereas birthing occurs mainly in summer (wet season, December–March; e.g., Solórzano and Cerdas 1989; Almeida-Santos and Salomão 2002; Valdujo et al. 2002; Monteiro et al. 2006; Nunes et al. 2010; Marques et al. 2013; Barros et al. 2014a,b; Almeida-Santos et al. 2017). Such conservatism might be determined by environmental and phylogenetic factors (Almeida-Santos and Salomão 2002; Barros et al. 2014b).

Bothrops atrox is a large, mainly nocturnal dietary generalist species of pit viper found throughout the Amazon rain forest, from eastern Colombia to northern Brazil, including Guyana, French Guyana, and Suriname, and also some regions of Peru, Venezuela, and Bolivia (Wüster et al. 1996; Oliveira and Martins et al. 2001; Martins et al. 2002; Campbell and Lamar 2004). This species inhabits areas with an equatorial climate (no autumn or winter) in which temperature and humidity variations are less marked than in regions inhabited by other species of *Bothrops* (e.g., Atlantic Forest, Cerrado, Caatinga, and Pantanal) and other North, Central, and South American crotalines studied so far (e.g., Aldridge and Duvall 2002; Monteiro et al. 2006; Barros et al. 2014a,b). Mating has been observed in January, April, and May (Martins and Oliveira 1998; Sanaiotti et al. 2005), whereas pregnancy and parturition have been reported from

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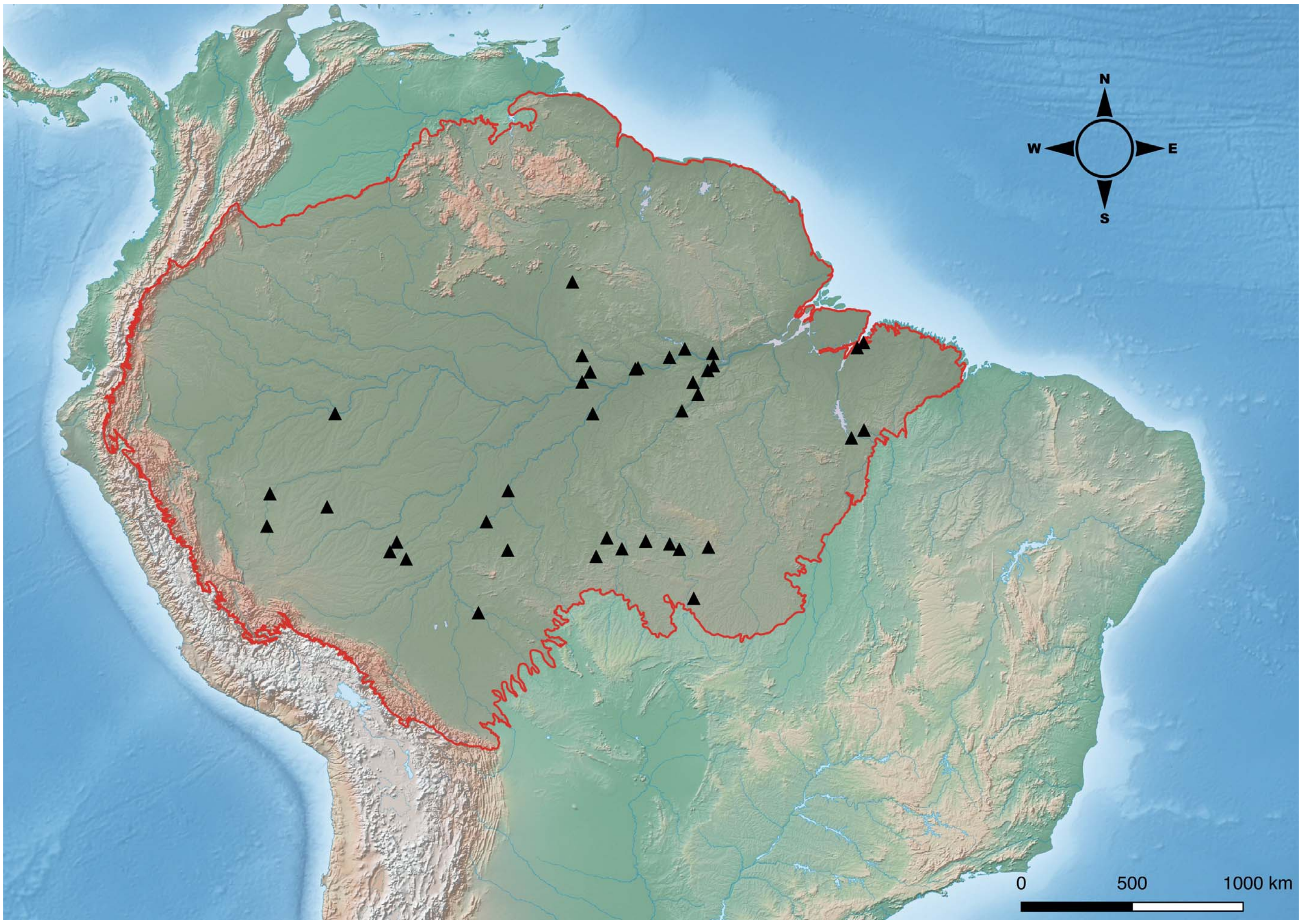

FIG. 1.—Geographic locations from where specimens of *Bothrops atrox* were collected in this study. The shaded area delimited in red represents the Amazon rain forest. A color version of this figure appears online.

May to December (Beebe 1946; Fitch 1970; Dixon and Soini 1986; Martins and Oliveira 1998). Although a few studies have commented about some aspects of the reproductive biology of *B. atrox*, this species was never evaluated in a focused study using histological evidence collected from comprehensive sampling. In relation to other pit vipers, *B. atrox* presents a contrasting model to investigate how environmental conditions can influence reproductive events, because the species occurs only in the areas influenced by the equatorial climate.

Here, using macroscopic and histological evidence from a broad sample of specimens, we describe the reproductive cycle of *B. atrox*, along with other characteristics of its reproductive biology (e.g., SSK, sperm storage, sexual maturity, dimorphism, and litter size). We also infer possible effects of the climate on some aspects of the reproductive cycle for this species in Amazonia.

MATERIALS AND METHODS

Morphological Data

We examined 339 individual *B. atrox* (78 female, 77 male, and 184 subadult specimens) collected throughout the Amazon rain forest in Brazil (Fig. 1) and preserved in scientific collections (Appendix I). Most of these individuals were collected from 1996 to 2013. We measured snout–vent length (SVL) and tail length (±1 mm) of each specimen before performing dissections and recording the sex through gonadal inspection. Females were considered sexually mature if they had secondary ovarian follicles, oviductal embryos, corpora lutea, or folded oviducts. Males were considered mature if they had convoluted ductus deferentia or spermatozoa in the reproductive tract. For adult females, we recorded the presence of corpora lutea and oviducts with emptied, but distinct, incubation chambers (indicative of postpartum condition); the number of ovarian follicles or oviductal embryos; and the diameter of the largest ovarian follicle (Almeida-Santos et al. 2014). For adult males, we recorded the diameter of the right and left distal ductus deferentia (between the kidney and the cloaca); length and width (in the proximal region) of the right kidney; and length, width, and thickness of the right testis. Urogenital organs were measured using a digital caliper (±0.1 mm).

Histological Data

Samples of the urogenital system from a subset of adult individuals were removed for histological examination. For females ($n = 26$), we excised the posterior portions of the right nonglandular uterus (see Siegel and Sever 2008). For males ($n = 56$), we collected the midregion of the testis, the

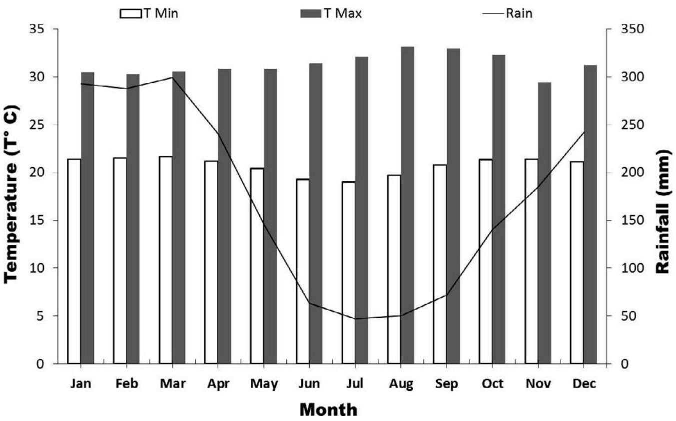

FIG. 2.—Monthly variation in temperature and rainfall in the Brazilian Amazon obtained from a combination of climatic data from the collection sites of the individuals examined in this study (see text for details). The wet season occurs from November to April, whereas the dry season occurs from May to October.

ductus deferentia (portion between the kidney and the cloaca), and the proximal region of the kidneys. Samples were stored in 70% ethanol until examination by light microscopy. After specimens were embedded using a standard paraffin method, we used a rotary microtome to produced transverse and longitudinal sections (5 μm), and stained them with hematoxylin and eosin (Junqueira and Carneiro 2013). Sections of the testes were assessed for sperm production. We classified spermatogenesis into one of the following six stages: Stage 1—testicular quiescence (seminiferous tubules with few spermatogonia and Sertoli cells; can contain few spermatozoa in the lumen); Stage 2—recrudescence (spermatogonia and primary spermatocytes appearing); Stage 3—spermiogenesis (metamorphosing spermatids); Stage 4—spermiation (mature spermatozoa in the lumen [maximal testicular activity]); Stage 5—late spermiation (spermatozoa abundant in the lumen, but spermatocytes and spermatids greatly reduced); and Stage 6—early regression (reduced germinal epithelium, with few spermatogonia, spermatocytes, and spermatids [spermatozoa might be present in the lumen; one or two layers of cells]).

Histological sections were photographed with an Olympus Q-Color 5 digital camera mounted on an Olympus BX51TF light microscope (Olympus Corp., Tokyo, Japan), and the images captured using Image-Pro Express software (v5, Media Cybernetics, Rockville, MD). We calculated the mean height of the seminiferous epithelium, the diameter of the seminiferous tubules, the height of the SSK epithelium, and the diameter of the SSK tubules by taking 6–10 measurements from each male using the ImageJ software v1.46 (Schneider et al. 2016).

Data Analysis

For the collection locality of each *B. atrox* specimen (Fig. 1), we obtained a climate profile using DIVA-GIS software (v7.1.7, https://www.diva-gis.org/) and the WorldClim v1.3 climate data set (from 1950 to 2000 with spatial resolution of 10 min; https://www.worldclim.org/). In general, areas within the Amazon rain forest have an equatorial climate with relatively constant mean temperatures throughout the year and well-marked seasonal rainfall (Fig. 2). We tested for seasonal variation in reproductive data by comparing variables between the wet season, November–April, and the dry season, May–October (Nobre et al. 2009).

Raw data were examined to ensure that statistical assumptions were met. Data were log-transformed when necessary to meet the assumptions appropriate for parametric statistical tests. Values are presented means ± 1 SD, and the significance level for all statistical tests was set at $\alpha < 0.05$. We tested for sexual size dimorphism in adults using a *t*-test, and for sexual dimorphism in tail length using analysis of covariance (ANCOVA), with SVL as the covariate. We determined litter size by counting the number of enlarged secondary follicles (>16 mm) or oviductal embryos in preserved specimens and the number of young born to females collected in nature and kept in captivity for short periods (1–6 mo). Linear regression was performed to determine the relationship between litter size and maternal SVL.

Data on captive parturitions were provided by researchers from several Amazonian states within the range of *B. atrox* (Acre in October and November; Pará in August and September; and Amazonas in June–October). The females from Acre were kept in the Laboratório de Herpetologia do Centro de Ciências Biológicas, da Universidade Federal do Acre, and were preserved in the herpetology collection of Universidade Federal do Acre (vouchers: 103, 588). The females from Pará were kept alive in the Laboratório de Pesquisas Zoológicas das Faculdades Integradas do Tapajós, and were preserved in the herpetology collection of the same laboratory (vouchers: 51, 25, 79, 84). The females ($n = 6$) from Amazonas were kept in the Instituto de Medicina Tropical de Manaus, Amazonas. Because the largest offspring born in captivity measured 254 mm SVL, we classified any preserved specimens with SVL less than this value as neonates.

We calculated the volume of the testes using the formula for an ellipse: testicular volume = $(4/3)\pi(abc/2)$; where a = anterior–posterior length, b = lateral width, and c = dorsal–ventral depth (Braz et al. 2014). Preliminary analyses indicated that male SVL was correlated with testicular volume ($r = 0.62$, $P = 0.0001$), kidney length ($r = 0.80$, $P = 0.0001$), and kidney width ($r = 0.42$, $P = 0.001$). Therefore, we tested for seasonal variation in these traits using ANCOVA (with SVL as the covariate). We used *t*-tests to evaluate seasonal variation in the diameter of the ductus deferens, the height of the seminiferous epithelium, the height of the SSK epithelium, and the diameter of the SSK tubules. Seasonal variation in the diameter of the seminiferous tubules was tested using a Mann–Whitney test because these data did not meet assumptions for parametric tests. Linear regressions were used to analyze the relationship between the independent variable of rainfall (mean monthly rainfall from WorldClim data set from 1950 to 2000) and five indicators of reproductive status (individual means of seminiferous tubule diameter, seminiferous epithelium height, SSK diameter, height of the SSK epithelium, and diameter of the largest secondary ovarian) as the dependent variables.

RESULTS

Sexual Maturity and Sexual Dimorphism

The smallest sexually mature male—having convoluted ductus deferentia with sperm in the lumen—measured 537

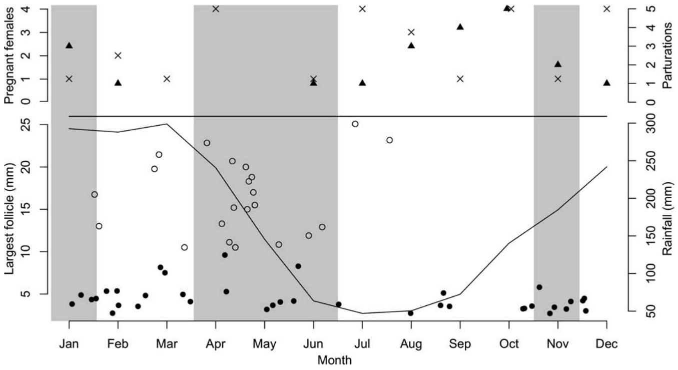

FIG. 3.—Reproductive cycle of female *Bothrops atrox* in Amazonian Brazil. Filled circles = primary vitellogenesis, open circles = secondary vitellogenesis, crosses = pregnant females, triangles = births. The line represents monthly variation in rainfall (wet season = November–April; dry season = May–October). Shaded areas indicate months with mating records.

mm SVL. Four male specimens having greater SVLs did not show evidence of having attained reproductive maturity. The smallest mature female measured 665 mm SVL and had eight developing embryos in the oviducts. Nine female specimens having greater SVLs did not show evidence of having attained reproductive maturity. Adult females averaged 939 ± 169 mm SVL (range = 665–1369 mm, $n = 60$), and adult males averaged 764 ± 139 mm SVL (range = 537–1116 mm, $n = 77$). Adult females were significantly larger than adult males ($t = 6.68$, df = 135, $P < 0.0001$), but adult males had longer tails relative to SVL than did females (ANCOVA, $F_{1,13} = 94.38$, $P < 0.0001$).

Female Reproduction

Mating was recorded in nature in April and May in the Reserva Florestal Adolpho Ducke, Manaus, Amazonas. Mating in November was observed at km 6 of the road to Iranduba, Amazonas. The mated female from November was in primary vitellogenesis (follicular size = 5.8 mm). An additional record of mating was observed in captivity (Instituto de Medicina Tropical de Manaus, Amazonas) in June. This mating occurred in the afternoon and lasted approximately 2 h (Appendix II).

Female specimens presented evidence of primary vitellogenesis occurring throughout the year, whereas evidence of secondary vitellogenesis occurred from January to August (mid-wet season to mid-dry season) but was most frequent in April (late wet season; Fig. 3). The diameter of the largest secondary follicle was not correlated with rainfall ($r = 0.02$, $P = 0.64$). Two females in secondary vitellogenesis (both collected in March) showed sperm in the nonglandular uterus (Fig. 4). Two females collected in April had secondary ovarian follicles simultaneously with oviductal eggs, indicating recent ovulation. Pregnant females were observed throughout the year (except May) and were evenly distributed across seasons ($\chi^2 = 0.77$, df = 3, $P = 0.86$; Fig. 3). However, both the timing of ovulation and pregnancy were asynchronous. For instance, two females collected in July and December had full-term embryos without residual yolk, and one female collected in October had early oviductal embryos with abundant yolk. Births in captivity and neonates preserved in museums occurred from June to February but were more frequently observed in the late dry season ($\chi^2 = 14.24$, df = 3, $P = 0.003$; Fig. 3). Litter size averaged 11 ± 8 young (range = 3–32, $n = 19$) and was positively correlated with female SVL ($r = 0.50$, $P = 0.001$, $n = 18$). Offspring SVL averaged 228 ± 23 mm (range = 156–254 mm, $n = 49$) and offspring tail length averaged 40 ± 5 mm (range = 27–50 mm, $n = 49$).

Male Reproduction

Different testicular stages were observed throughout the year (Figs. 5 and 6). Testicular quiescence (Stage 1) was observed from April to July (late wet season to mid-dry season). Testicular recrudescence (Stage 2) was observed in July (dry season). Spermiogenesis (Stage 3) was observed for most of the wet season (November–April). Spermiation (Stage 4) occurred during the late dry season (October) and throughout the entirety of the wet season (November–April).

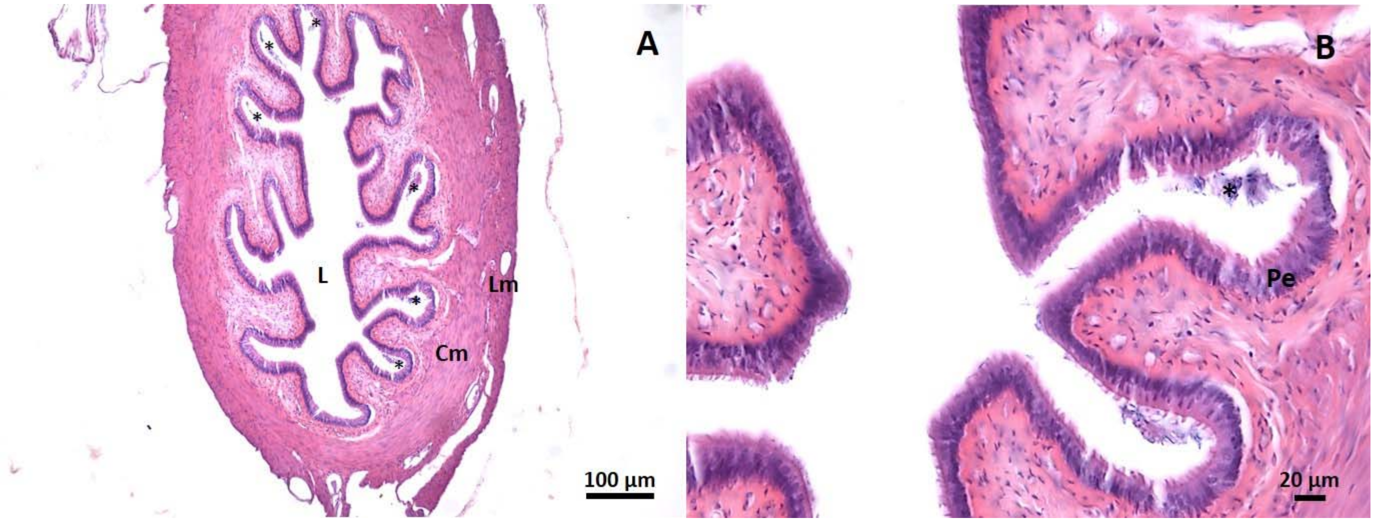

FIG. 4.—Sperm storage in the nonglandular uterus of female *Bothrops atrox*. (A) Sperm stored in the nonglandular uterus of a female collected in March. (B) Folds of the nonglandular uterus with sperm stored. L = lumen, Cm = circular muscle, Lm = longitudinal muscle, Pe = pseudostratified columnar epithelium composed of interspersed ciliated and secretory cells. Asterisk indicates spermatozoa in the folds of the nonglandular uterus. A color version of this figure appears online.

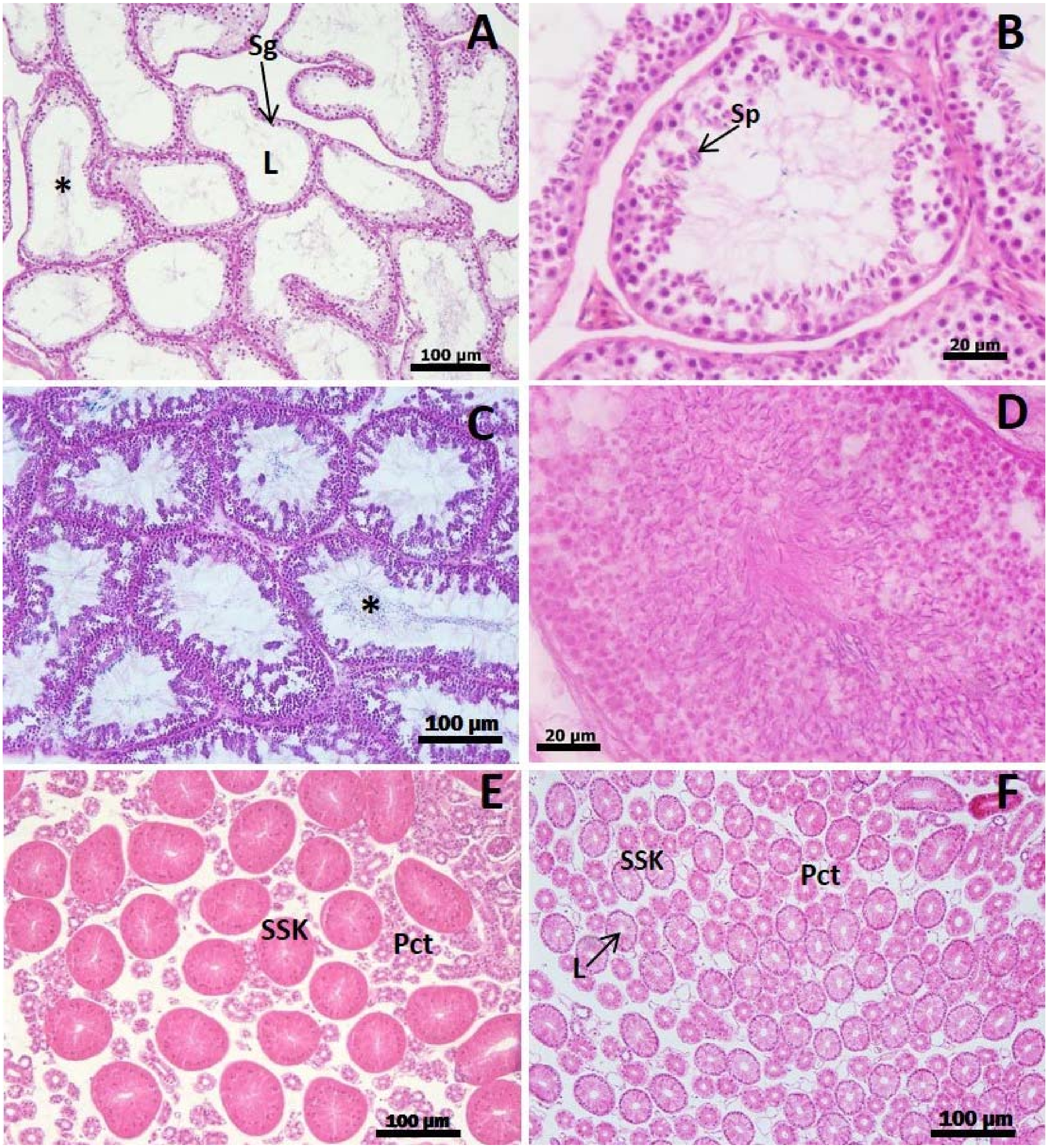

FIG. 5.—Representative histological sections of the testes and sexual segment of the kidney (SSK) of *Bothrops atrox*. (A) Testis in complete regression in the dry season. (B) Testis in spermiogenesis in the wet season. (C) Testis in spermiation in the wet season. (D) Testis in spermiation (maximal testicular activity). (E) SSK hypertrophy in the wet season. (F) SSK in regression in the dry season. Asterisks indicate spermatozoa in the lumen. L = lumen, Sg = spermatogonia, Sp = spermatocyte in metamorphosis, Pct = proximal convoluted tubule. A color version of this figure appears online.

Late spermiation (Stage 5) occurred in May (early dry season) and October–November (early wet season). Finally, early regression (Stage 6) was observed in March (late wet season) and September (late dry season). There was no correlation between rainfall and seminiferous tubule diameter ($r = 0.12$, $P = 0.45$), seminiferous epithelium height ($r = 0.06$, $P = 0.71$), or testicular volume ($r = 0.08$, $P = 0.07$).

Both the seminiferous tubule diameter (Mann–Whitney U-test, $U = 90.0$, $P = 0.003$) and testicular volume (ANCOVA, $F_{1,59} = 5.78$, $P = 0.02$; Table 1; Fig. 7) were

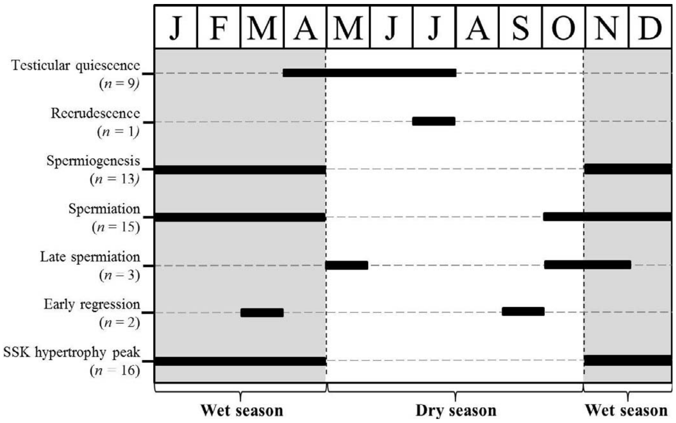

FIG. 6.—Timing of the spermatogenic stages and cycle of the sexual segment of the kidney (SSK) in *Bothrops atrox*. Letters across the top of panel represent the calendar months. See Methods for definitions of the spermatogenic stages.

greater in the wet season than in the dry season. Both the seminiferous epithelium height ($t = 1.52$, df $= 38$, $P = 0.13$; Table 1) and the diameter of the ductus deferentia ($t = 1.10$, df $= 64$, $P = 0.30$; Fig. 7) were similar between seasons. Sperm were observed in the ductus deferentia throughout the year (Fig. 8). The SSK was secretory and hypertrophied throughout the year, but SSK diameter ($t = 2.91$, df $= 41$, $P = 0.006$) and epithelial height ($t = 3.86$, df $= 41$, $P < 0.001$) were greater in the wet season (November–April; Table 1; Fig. 5e). Moreover, both variables were positively correlated with rainfall (SSK diameter: $r = 0.36$, $P = 0.02$; epithelial height: $r = 0.39$, $P = 0.008$). Kidney length (ANCOVA, $F_{1,56} = 1.92$, $P = 0.17$) and kidney width ($F_{1,51} = 1.62$; $P = 0.21$; Fig. 7) were similar between seasons.

DISCUSSION

Sexual Dimorphism, Sexual Maturity, and Fecundity

Female *Bothrops* are larger and attain sexual maturity at a larger body size than males (Sazima 1992; Hartmann et al. 2004; Monteiro et al. 2006; Nunes et al. 2010; F.M. Silva et al. 2017). Late sexual maturity and larger body sizes in females are advantageous because they enable the production of larger litters (Shine 1993, 1994). This is true for *B. atrox* because litter size increases with female body size. Considering the species of the *B. atrox* group, the litter size of *B. atrox* (3–32 young) is similar to that of *B. moojeni* (3–32 young; Nogueira et al. 2003) and *B. leucurus* (3–29 young; Barros et al. 2014b), but smaller than that of *B. asper* (5–86 young; Solórzano and Cerdas 1989; Sasa et al. 2009), perhaps because of the larger body size of *B. asper*.

Males are generally larger than females in species exhibiting male–male combat (Shine 1994; Aldridge and Duvall 2002). This is not the case for the *B. atrox* complex, however, which is the only group of *Bothrops* in which there is male–male combat and females are larger than males. Male–male combat has been reported in *B. atrox*, *B. moojeni*, and *B. leucurus* (Almeida-Santos and Salomão 2002; Almeida-Santos et al. 2017), and females of these species are larger than males (Nogueira et al. 2003; Almeida-Santos et al. 2017; F.M. Silva et al. 2017). However, the maximum body size of males of the *B. atrox* group (range = 1060–1270 mm SVL; Nogueira et al. 2003; Barros et al. 2014b) is larger than males of many other species of *Bothrops* that lack male combat (ranging from 645–980 mm SVL; Hartmann et al. 2004; Monteiro et al. 2006; Nunes et al. 2010; Marques et al. 2013; Barros et al. 2014a). We suggest that the origin of male–male combat favored the evolution of larger male body size in the *B. atrox* group compared with males of other species of *Bothrops*.

Reproductive Cycles

The occurrence of pregnant females and parturition events in almost every month of the year indicates that the reproductive cycle of *B. atrox* is more extended and more variable than that of other pit vipers. Pregnancies and births of other pit vipers are restricted to one or two seasons, more specifically in spring or summer (Aldridge and Duvall 2002; Almeida-Santos and Salomão 2002; Barros et al. 2012). The ovulation period might also be different for the *B. atrox–B. asper* group (sensu Saldarriaga-Córdoba et al. 2017). These species might ovulate in the same months in which copulation occurs (April, May, and July; Nogueira et al. 2003; Sasa et al. 2009; Barros et al. 2014b), so long-term sperm storage is likely to be facultative in the *B. atrox* group. For other pit vipers, ovulation rarely occurs during the copulation season and sperm storage is obligatory for pregnancy to occur in the hottest season of the year. Furthermore, mating in crotalines is commonly synchronous with secondary vitellogenesis (Aldridge and Duvall 2002; Almeida-Santos and Salomão 2002). Female *B. atrox* can also mate during primary vitellogenesis, however, for which sperm storage is a fundamental component of the female reproductive cycle. Thus, mating of *B. atrox* in primary vitellogenesis needs further investigation to understand the mechanisms of attractiveness of females, because the deposition of yolk in the follicles was not initiated by means of vitellogenin, which acts in the process of estrous signaling (Crews and Garstka 1982; Price 2017).

Although pregnant *B. atrox* were found throughout the year, vitellogenesis and the frequency of birth were seasonal. In *B. atrox*, the vitellogenic cycle was temporally focused during the transition period between the wet and dry seasons (April), and coincided with sperm storage and the mating season. In other crotalines, vitellogenesis occurs over a relatively extended period (e.g., *B. erythromelas* from the semiarid Caatinga; Barros et al. 2014a) or interrupted during the winter (e.g., *Crotalus viridis* from temperate prairies; Aldridge 2002). Temperature, humidity, and availability of

TABLE 1.—Seasonal variation in microscopic measurements (µm) of the urogenital structures of male *Bothrops atrox* collected in Amazonian Brazil. Data are expressed as mean ± 1 SD (range). Except for seminiferous epithelial height, all variables differed between dry and wet seasons (*t*-tests, $P < 0.05$).

Season	*n*	Seminiferous tubule diameter	Seminiferous epithelial height	Sexual segment of kidney (SSK) diameter	SSK epithelial height
Dry	16	149 ± 36 (87–215)	39 ± 34 (10–134)	90 ± 18 (59–122)	36 ± 9 (26–55)
Wet	27	200 ± 74 (101–490)	57 ± 36 (11–198)	112 ± 26 (68–157)	50 ± 12 (27–70)

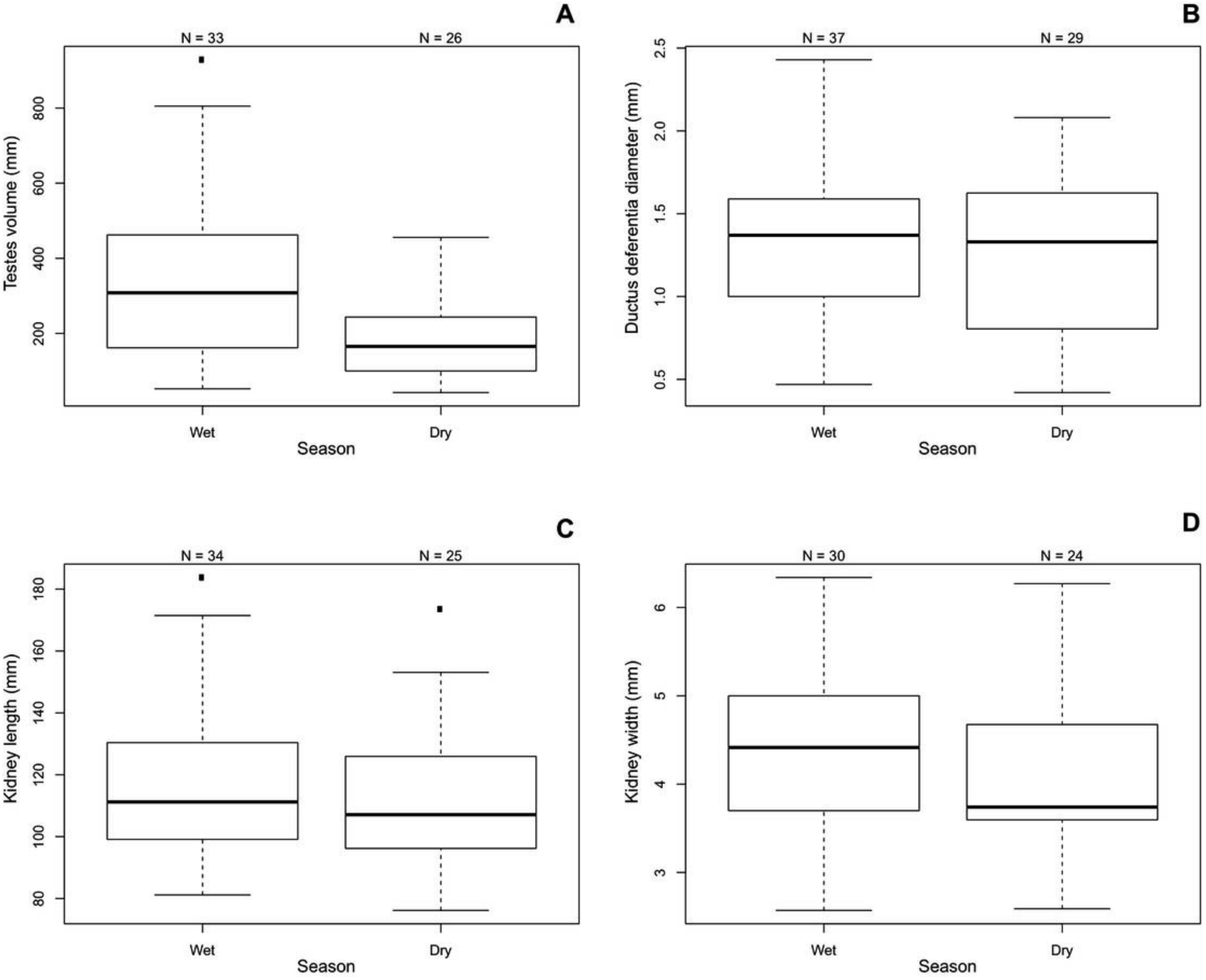

FIG. 7.—Seasonal variation in urogenital structures of male *Bothrops atrox*. (A) Testis volume, (B) ductus deferens diameter, (C) kidney length, and (D) kidney width. Middle line represents mean values, boxes show standard errors, whiskers represent the minimum and maximum values, black squares indicate outliers.

prey of the Amazon might all favor a shorter vitellogenic cycle (Bonnet et al. 2001; Aldridge and Duvall 2002). Regarding the availability of prey, *B. atrox* probably does not experience months without food because of its generalist diet in a habitat with abundant prey resources (Martins and Oliveira 1998; Martins et al. 2002; Bernarde and Abe 2010; K.M.P. Silva et al. 2017).

Seasonal reproduction seems to be common among snake species of subtropical, tropical, and equatorial regions; synchrony among individuals, however, might not be as pronounced (Mathies 2011). Two factors might explain the interindividual asynchrony of pregnancy in *B. atrox*. The first factor is hydrophysical—a combination of temperature and humidity—which is relatively constant throughout the year. Unlike many crotalines, wherein pregnancy occurs during the warmest period of the year (Aldridge and Duvall 2002), ovulation, fertilization, and pregnancy in *B. atrox* can occur at any time of the year. High temperatures, such as those in the Amazon rain forest, enhance embryonic development (Vinegar 1974; Hubert 1985). Moreover, pregnancy and parturition are synchronous in female *Bothrops* inhabiting areas with high mean annual temperatures and long periods of drought. For instance, pregnant *B. pauloensis* (of the Cerrado) are found from June to December (mid-dry season to mid-wet season), and births occur in the wet season (Valdujo et al. 2002). Pregnant *B. erythromelas* (of the Caatinga) are found during spring (wet season), and births occur in spring and summer (wet season; Barros et al. 2014a). The other factor explaining the interindividual asynchrony of pregnancy in *B. atrox* is sperm storage. Females in primary vitellogenesis or at the beginning of secondary vitellogenesis would copulate and store sperm until they have sufficient energy reserves to complete the vitellogenic cycle.

Parturition in many pit vipers, including *Agkistrodon, Bothrops, Crotalus, Porthidium,* and *Trimeresurus*, occurs in the warmest or wettest seasons (Solórzano 1990; McCoy and Censky 1992; Tsai and Tu 2001; Aldridge and Duvall 2002;

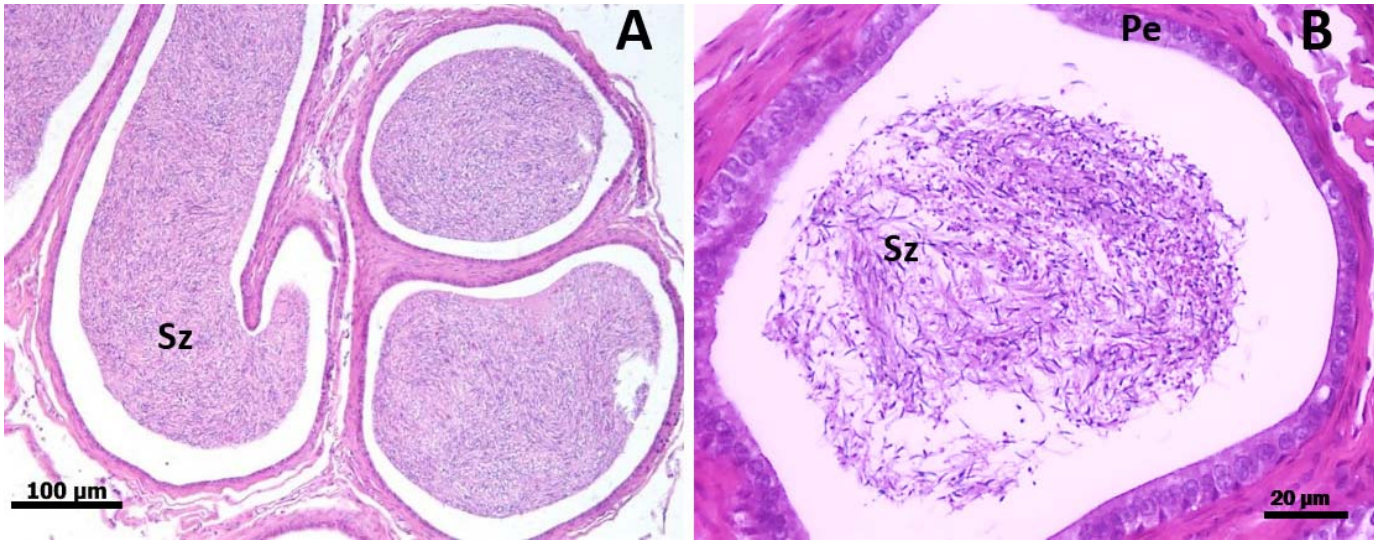

FIG. 8.—Representative histological sections of ductus deferentia of male *Bothrops atrox*. (A) Ductus deferens packed with sperm in April (late wet season), (B) ductus deferens with sperm stored in May (early dry season). Sz = spermatozoa, Pe = pseudostratified epithelium. A color version of this figure appears online.

Almeida-Santos and Salomão 2002; Barros et al. 2012). In *B. atrox*, however, births occur almost year-round, but are more frequent from August to October (late dry season). In addition, individuals of *B. atrox* are more frequently encountered around streams and, during those months, the soils are not yet flooded (Fraga et al. 2013), which might make it easier for females to locate safe sites for parturition (i.e., enhancing offspring survival; Brown and Shine 2006).

The testicular quiescence observed in *B. atrox* characterizes the cycle of individual males as discontinuous (Mathies 2011). At the population level, the spermatogenic cycle of *B. atrox* is seasonal semisynchronous (Mathies 2011), with spermiation occurring in the wet season. In general, the male reproductive cycle of the species of the *B. atrox* group is seasonal (Barros et al. 2014b; Almeida-Santos et al. 2017). Spermatogenesis peaks in the wet season for *B. atrox* and *B. moojeni* from the Cerrado, although for the former it occurs from November to April and for the latter December to March (Almeida-Santos et al. 2017). Spermatogenesis peaks within the wet season for both *B. atrox* and *B. moojeni* in the Cerrado ecoregion from November to April and December to March, respectively (Almeida-Santos et al. 2017). In *B. leucurus* from the Atlantic Forest, spermatogenesis occurs during the late wet season (December–March) and early dry season (April–June; Barros et al. 2014b). Thus, as reported for other pit vipers (Aldridge and Duvall 2002), spermatogenesis in *B. atrox* usually occurs in the hottest and wettest seasons.

For temperate-zone crotalines, temperature and rainfall seem to be the most influential abiotic factors modulating the seasonality of the male reproductive cycle (Aldridge 1975; Lutterschmidt and Mason 2009). The seasonal spermatogenic cycle of pit vipers from temperate zones begins in late spring or early summer and peaks in midsummer (August–September; Saint-Girons 1982) or until autumn (Johnson et al. 1982; Aldridge and Duvall 2002). Although temperatures are constantly high throughout the year in the Amazon, rainfall seems to exert a strong influence on the reproductive cycle of *B. atrox*. The wet season of the Amazon directly influences the activity of *B. atrox*, which is among the most commonly encountered snake species in the field (Oliveira and Martins 2001). The increased incidence of snakes is probably attributable to increased availability of some types of prey (e.g., anurans; Oliveira and Martins 2001; Bernarde and Abe 2010).

Among crotalines, spermiogenesis and the mating season are generally synchronized with SSK hypertrophy, as in *Agkistrodon piscivorus* (Johnson et al. 1982), *B. atrox*, *B. erythromelas* (Barros et al. 2014a), *C. horridus* (Aldridge and Brown 1995), *C. viridis viridis* (Aldridge 1993), *C. v. helleri*, and *C. v. oreganus* (Aldridge 2002). Furthermore, SSK hypertrophy in *B. atrox* and *C. viridis* coincides with the timing of male–male combat (Aldridge 2002). SSK cells are androgen dependent, and so SSK hypertrophy is caused by increased testosterone levels (Fox 1977; Krohmer et al. 2004; Sever and Hopkins 2005; Rojas et al. 2013). SSK secretions have been hypothesized to have different functions, including sperm nutrition, sperm activation in the female reproductive tract, copulatory plug formation, and maintenance of stored sperm (Marinho et al. 2009; Aldridge et al. 2011; Friesen et al. 2013; Barros et al. 2017).

In *B. atrox*, SSK hypertrophy, spermiogenesis, and a higher number of vitellogenic females were synchronous with mating in January–April. By storing sperm in the ductus deferens and oviduct, males and females, respectively, are capable of mating at any gonadal stage, thus facilitating their reproductive success. This suggestion is supported by our observations of mating in May and June (months in which testes are regressed or completely quiescent) and in November (when females are in primary vitellogenesis).

The reproductive cycles of male and female *B. atrox* appear to be most influenced by precipitation and temperature. Males show SSK hypertrophy, spermiogenesis, and combat in the wet season. Females show follicular recrudescence in the wet season, pregnancy from January to December, and parturition events concentrated in the late dry season. Both males and females store sperm in their reproductive tract as an alternative reproductive strategy.

Collectively, our results show that *B. atrox* has unique reproductive strategies that confer greater reproductive plasticity to this species compared with other pit vipers that have been thus studied.

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Literature Cited

Aldridge, R.D. 1975. Environmental control of spermatogenesis in the rattlesnake, *Crotalus viridis*. Copeia 1975:493–496.

Aldridge, R.D. 1993. Male reproductive anatomy and seasonal occurrence of mating and combat behavior of the rattlesnake (*Crotalus v. viridis*). Journal of Herpetology 27:481–484.

Aldridge, R.D. 2002. The link between mating season and male reproductive anatomy in the rattlesnakes *Crotalus viridis oreganus* and *C. v. helleri*. Journal of Herpetology 36:295–300.

Aldridge, R.D., and W.S. Brown. 1995. Male reproductive cycle, age at maturity, and cost of reproduction in the timber rattlesnake (*Crotalus horridus*). Journal of Herpetology 29:399–407.

Aldridge, R.D., and D. Duvall. 2002. Evolution of the mating season in the pitvipers of North America. Herpetological Monographs 16:1–25.

Aldridge, R.D., B.C. Jellen, D.S. Siegel, and S.S. Wisniewski. 2011. The sexual segment of the kidney. Pp. 477–509 in Reproductive Biology and Phylogeny of Snakes (R.D. Aldridge and D.M. Sever, eds.). Science Publishers, USA.

Almeida-Santos, S.M., and M.G. Salomão. 2002. Reproduction in neotropical pitvipers, with emphasis on species of the genus *Bothrops*. Pp. 445–462 in Biology of the Vipers (G.W. Schuett, M. Höggren, M.E. Douglas, and H.W. Greene, eds.). Eagle Mountain Publishing, USA.

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

Almeida-Santos, S.M., V.A. Barros, C.A. Rojas, L.R. Sueiro, and R.H.C. Nomura. 2017. Reproductive biology of the Brazilian lancehead, *Bothrops moojeni* (Serpentes, Viperidae), from the state of São Paulo, Southeastern Brazil. South American Journal of Herpetology 12:174–181.

Barros, V.A., L.R. Sueiro, and S.M. Almeida-Santos. 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., C.A. Rojas, and S.M. Almeida-Santos. 2014a. Reproductive biology of *Bothrops erythromelas* from the Brazilian Caatinga. Advances in Zoology 2014:1–11.

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

Barros, V.A., C.A. Rojas, K.M.P. Silva, and S.M. Almeida-Santos. 2017. Natural history notes: *Bothrops pubescens* (sperm in the sexual segment of the kidneys). Herpetological Review 48:666–667.

Beebe, W. 1946. Field notes on the snakes of Kartabo, British Guiana and Caripito, Venezuela. Zoologica 31:11–52.

Bernarde, P.S., and A.S. Abe. 2010. Hábitos alimentares de serpentes em Espigão do Oeste, Rondônia, Brasil. Biota Neotropica 10:167–173.

Bonnet, X., G. Naulleau, R. Shine, and O. Lourdais. 2001. Short-term versus long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis*. Oikos 92:297–308.

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

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

Campbell, J.A., and W.W. Lamar. 2004. The Venomous Reptiles of the Western Hemisphere. Comstock Publishing, USA.

Crews, D., and W.R. Garstka. 1982. The ecological physiology of a garter snake. Scientific American 247:158–171.

Dixon, J.R., and P. Soini. 1986. The Reptiles of the Upper Amazon Basin, Iquitos Region, Peru. Milwaukee Public Museum, USA.

Fitch, H.S. 1970. Reproductive cycles in lizards and snakes. Miscellaneous Publications of the Museum of Natural History, University of Kansas 52:1–247.

Fitch, H.S. 1982. Reproductive cycles in tropical reptiles. Occasional Papers of the Museum of Natural History, University of Kansas 96:1–53.

Fraga, R., W.E. Magnusson, C.R. Abrahão, T. Sanaiotti, and A.P. Lima. 2013. Habitat selection by *Bothrops atrox* (Serpentes: Viperidae) in Central Amazonia, Brazil. Copeia 2013:684–690.

Friesen, C.R., R. Shine, R.W. Krohmer, and R.T. Mason. 2013. Not just a chastity belt: The functional significance of mating plugs in garter snakes, revisited. Biological Journal of the Linnean Society 109:893–907.

Fox, H. 1977. The urogenital system of reptiles. Pp. 1–157 in Biology of the Reptilia, vol. 6 (C. Gans and T.S. Parsons, eds.). Academic Press, USA.

Hartmann, M.T., O.A.V. Marques, and S.M. Almeida-Santos. 2004. Reproductive biology of the southern Brazilian pitviper *Bothrops neuwiedi pubescens* (Serpentes, Viperidae). Amphibia–Reptilia 25:77–85.

Hubert, J. 1985. Embryology of the Squamata. Pp. 1–55 in Biology of the Reptilia, vol. 15 (C. Gans and F. Billet, eds.). Wiley and Sons, USA.

Johnson, L.F., J.S. Jacob, and P. Torrance. 1982. Annual testicular and androgenic cycles of the cottonmouth (*Agkistrodon piscivorus*) in Alabama. Herpetologica 38:16–25.

Junqueira, L.C., and J. Carneiro. 2013. Histologia Básica: Texto e Atlas. Guanabara Koogan, Brasil.

Krohmer, R.W., and D.I. Lutterschmidt. 2011. Environmental and neuroendocrine control of reproduction in snakes. Pp. 290–346 in Reproductive Biology and Phylogeny of Snakes (R.D. Aldridge and D.M. Sever, eds.). Science Publishers, USA.

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

Lutterschmidt, D.I., and R.T. Mason. 2009. Endocrine mechanisms mediating temperature-induced reproductive behavior in red-sided garter snakes (*Thamnophis sirtalis parietalis*). Journal of Experimental Biology 212:3108–3118.

Marinho, C.E., S.M. Almeida-Santos, S.C. Yamasaki, and P.F. Silveira. 2009. Peptidase activities in the semen from the vas deferens and uterus of the neotropical rattlesnake *Crotalus durissus terrificus*. Journal of Comparative Physiology 179:635–642.

Marques, O.A.V., K. Kasperoviczus, and S.M. Almeida-Santos. 2013. Reproductive ecology of the threatened pitviper *Bothrops insularis* from Queimada Grande Island, Southeast Brazil. Journal of Herpetology 47:393–399.

Martins, M.M., and M.E. Oliveira. 1998. Natural history of snakes in forests of the Manaus region, central Amazonia, Brazil. Herpetological Natural History 6:78–150.

Martins, M., O.A.V. Marques, and I. Sazima. 2002. Ecological and phylogenetic correlates of feeding habits in neotropical pitvipers of the genus *Bothrops*. Pp. 307–328 in Biology of the Vipers (G.W. Schuett, M. Höggren, M.E. Douglas, and H.W. Greene, eds.). Eagle Mountain Publishing, USA.

Mathies, T. 2011. Reproductive cycles of tropical snakes. Pp. 511–550 in Reproductive Biology and Phylogeny of Snakes (R.D. Aldridge and D.M. Sever, eds.). Science Publishers, USA.

McCoy, C.J., and E.J. Censky. 1992. Biology of the Yucatan hognosed viper, *Porthidium yucatanicum*. Pp. 217–222 in Biology of the Pitvipers (J.A. Campbell and E.D. Brodie, Jr., eds.). Selva, USA.

Monteiro, C., C.E. Montegomery, F. Spina, R.J. Sawaya, and M. Martins. 2006. Feeding, reproduction, and morphology of *Bothrops mattogrossensis* (Serpentes, Viperidae, Crotalinae) in the Brazilian Pantanal. Journal of Herpetology 40:408–413.

Nobre, C.A., G.O. Obregón, J.A. Marengo, R. Fu, and G. Poveda. 2009. Characteristics of Amazonian climate: Main features. Geophysical Monograph Series 1:149–162.

Nogueira, C., R.J. Sawaya, and M. Martins. 2003. Ecology of the pitviper, *Bothrops moojeni*, in the Brazilian Cerrado. Journal of Herpetology 37:653–659.

Nunes, S.F., I.L. Kaefer, P.T. Leite, and S.Z. Cechin. 2010. Reproductive and feeding biology of the pitviper *Rhinocerophis alternatus* from subtropical Brazil. Herpetological Journal 20:31–39.

Oliveira, M.E., and M. Martins. 2001. When and where to find a pitviper: Activity patterns and habitat use of the lancehead, *Bothrops atrox*, in central Amazonia, Brazil. Herpetological Natural History 8:101–110.

Price, E.R. 2017. The physiology of lipid storage and use in reptiles. Biological Reviews 92:1406–1426.

Rojas, C.A., V.A. Barros, and S.M. Almeida-Santos. 2013. The reproductive cycle of the male sleep snake *Sibynomorphus mikanii* (Schlegel, 1837) from southeastern Brazil. Journal of Morphology 274:215–228.

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

Saldarriaga-Córdoba, M., C.L. Parkinson, J.M. Daza, W. Wuster, and M. Sasa. 2017. Phylogeography of the Central American lancehead *Bothrops asper* (Serpentes: Viperidae). Plos One 12:1–20.

Sanaiotti, T.M., J.D. Do Vale, G. Bivati, and Z. Campos. 2005. Natural history notes: *Bothrops atrox* (mating). Herpetological Review 36:321–322.

Sasa, M., D.K. Wasko, and W.W. Lamar. 2009. Natural history of the terciopelo *Bothrops asper* (Serpentes: Viperidae) in Costa Rica. Toxicon 54:904–922.

Sazima, I. 1992. Natural history of the jararaca pitviper, *Bothrops jararaca,* in southeastern Brazil. Pp. 199–216 in Biology of the Pitvipers (J.A. Campbell and E.D. Brodie, eds.). Selva, USA.

Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2016. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

Sever, D.M., and W.A. Hopkins. 2005. Renal sexual segment of the ground skink, *Scincella laterale* (Reptilia, Squamata, Scincidae). Journal of Morphology 266:46–59.

Shine, R. 1993. Sexual dimorphism in snakes. Pp. 49–86 in Snakes: Ecology and Behaviour (R.A. Seigel and J.T. Collins, eds.). McGraw-Hill, USA.

Shine, R. 1994. Sexual dimorphism in snakes revisited. Copeia 1994:326–346.

Siegel, D.S., and D.M. Sever. 2008. Seasonal variation in the oviduct of female *Agkistrodon piscivorus* (Reptilia: Squamata): An ultrastructural investigation. Journal of Morphology 269:980–997.

Siegel, D.S., D.M. Sever, J.L. Rheubert, and K.M. Gribbins. 2009. Reproductive biology of *Agkistrodon piscivorus* Lacépède (Squamata, Serpentes, Viperidae, Crotalinae). Herpetological Monographs 23:74–107.

Silva, F.M., L.S. de Oliveira, L.R.S. Nascimento, F.A. Machado, and A.L.C. Prudente. 2017. Sexual dimorphism and ontogenetic changes of Amazonian pit vipers (*Bothrops atrox*). Zoologischer Anzeiger 271:15–24.

Silva, K.M.P., S.M. Almeida-Santos, and R. Bertani. 2017. Hundred legs good, two fangs better: Adult centipede (Scolopendridae) devoured by a juvenile Amazon lancehead, *Bothrops atrox* (Viperidae). Acta Amazonica 47:171–174.

Solórzano, A. 1990. Reproduction in the pit viper *Porthidium picadoi* Dunn (Serpentes: Viperidae) in Costa Rica. Copeia 1990:1154–1157.

Solórzano, A., and L. Cerdas. 1989. Reproductive biology and distribution of the terciopelo, *Bothrops asper* Garman (Serpentes: Viperidae), in Costa Rica. Herpetologica 45:444–450.

Tsai, T.S., and M.C. Tu. 2001. Reproductive cycle of female Chinese green tree vipers, *Trimeresurus stejnegeri stejnegeri*, in Northern Taiwan. Herpetologica 57:157–168.

Valdujo, P.H., C.C. Nogueira, and M. Martins. 2002. Ecology of *Bothrops neuwiedi pauloensis* (Serpentes: Viperidae: Crotalinae) in the Brazilian Cerrado. Journal of Herpetology 36:169–57.

Vinegar, A. 1974. Evolutionary implications of temperature induced anomalies of development in snake embryos. Herpetologica 30:72–74.

Wüster, W., R.S. Thorpe, G. Puorto, M.F.D. Furtado, S.A. Hoge, M.G. Salomão, R.D.G. Theakston, and D.A. Warrell. 1996. Systematics of the *Bothrops atrox* complex (Reptilia: Serpentes: Viperidae) in Brazil: A multivariate analysis. Herpetologica 52:263–271.

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APPENDIX I

Specimens Examined

Collection of amphibians and reptiles of the Instituto Nacional de Pesquisas da Amazônia (INPA).—31484, 11906, 25886, 28796, 30292, 31509, 31514, 31508, 14736, 18070, 28797, 28590, 9689, 31517, 31551, 31487, 1235, 31486, 31516, 20375, 31490, 19808, 31551, 9688, 31510, 31511, 1145, 31487, 1235, 31491, 31486, 31515, 10933, 20375. The regions sampled included the states of Amazonas and Pará.

Laboratory of Zoology of the Faculdades Integradas do Tapajós (LPZ).—1530, 1729, 2585, 5556, 1283, 5554, 1728, 1674, 1920, 5557, 4898, 3319, 3170, 1117, 2680, 1084, 1251, 3597, 5503, 2808, 2236, 2224, 3796, 1112, 2684, 2760, 3679, 5627, 5628, 5142, 5600, 5636, 5635, 5787, 5783, 2655, HMC 263, 4298, HMC 27, HMC 78, 2583, 4302, 980, 1789, 2763, 5558, 401, 2729, 5550, 5551, 1223, 1793, 3633, 4517, 2766, 3642, 2815, 2531, 1033, 2309, 5777, 5776, 289, 5549, HMC 195, HMC 281, HMC 60, HMC 176, HMC 26, HMC 193, HMC 42, 4225, HMC 188, HMC 141, 5549, 5615, 970, 1208, 1265, 1207, 1424, 1336, 5555, 5501, 3027, 5504, 4900, 1984, 2691, 5568, 5502, 1651, 1498, 2665, 2666, 2222, 2821, 1306, 1305, 1921, 1643, 3600, 3313, 2838, 5634, 4906, 4903, 5637, 5742, 4839, 576, 396, HMC 66, HMC 180, HMC 283, HMC 84, HMC 62, HMC 157, HMC 65, HMC 108, HMC 106, HMC 124, HMC 261, HMC 57, HMC 55, HMC 282, HMC 138, HMC 7. Pará was the only sampled state.

Museu Nacional (MNRJ).—16822, 15008, 958, 3003, 967, 3411, 16824, 17965, 17966, 14244, 16825, 16821, 15003, 23280, 3002, 8415, 974, 8577, 16823, 16070, 16505, 23217, 20294, 17952, 349, 20663, 16510, 19833, 16820, 18950, 16098, 18945, 18944, 8301, 959, 963, 960, 9015, 2990, 10049, 8576, 10835, 3031, 3016, 3015, 3407, 8300, 3409, 1294, 2764, 2765, 17967. The regions sampled included the states of Amazonas, Pará, Rondônia, and Mato Grosso.

Museum of science and technology of the Pontifícia Universidade Católica do Rio Grande do Sul (MCT).—8896, 7922, 8898, 6369, 14576, 11326, 8909, 7625, 14578, 7921. Pará was the only sampled state.

Herpetology collection of the Universidade Federal do Acre; (UFAC).—16, 255, 336, 168, 30235, 36, 39, 351, 32, 350, 169, 353, 207, 209, 55, 52, 125, 117, 14, 38, 116, 17, 81, 337, 4, 34, 33, 53. Acre was the only sampled state.

Herpetology collection of the Universidade Federal do Mato Grosso (UFMT).—7223, 7228, 6220, 7778, 8232, 8907, 8915, 8581, 7704, 7204, 8570, 8577, 8547, 8160, 8444, 5718, 7774, 8550, 6424, 6812, 8924, 8580, 8899. Mato Grosso was the only sampled state.

Herpetological reference collection from the state of Rondônia of the Universidade Federal de Rondônia (CRHRO).—165, 2569, 284, 19873, 151, 155, 159, 2573, 163, 1304, 154, 161, 167, 164, 148, 8499, 169, 153, 158, 162, 156, 152, 160, 157, 2575, 466, 2018, 168, 2572. Rondônia was the only sampled state.

Museum of Zoology of the Universidade Estadual de Campinas (ZUEC).—1635, 1633, 169, 1974, 2045, 1604. Acre was the only sampled state.

APPENDIX II

Mating in Nature

April, wet season.—(1) Couple was found on dirt road; A.C. Weber, personal observation. (2) Couple was found inside Igarapé, 1 m wide and 5–10 cm deep, between 0900 and 1000 h; S.N. Oliveira, personal observation. (3) Couple found in copulation at 0936 h; R.C. Vogt, personal observation. (4) Couple was found in Mata trail, at the Universidade Federal do Amazonas at 0900 h; R. Silveira, personal observation.

November, early wet season.—Couple was preserved in Coleção Zoológica Paulo Burheim-répteis, voucher of female CZPB-RP 497, voucher of male CZPB-RP 496.