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# Is rainfall seasonality important for reproductive strategies in viviparous Neotropical pit vipers? A case study with *Bothrops leucurus* from the Brazilian Atlantic Forest

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Two populations of the Neotropical lancehead *Bothrops leucurus* were studied in two locations in Brazil (Espírito Santo, ES, and Bahia, BA) with different rainfall seasonality patterns. The timing of reproduction was very similar in both populations, with the mating season occurring in autumn (when spermatozoa were found in uteri) and births occurring in summer. In males, spermatogenesis peaked in autumn, with evidence for increased secretory activity in the epithelium of the ductus deferens during the mating season in both populations. Our results indicate that phylogenetic inertia plays a major role in determining the timing of reproductive events in *B. leucurus*. However, snout-vent length (SVL) and clutch size were larger in individuals from BA than ES, which may be a result of differences in rainfall seasonality or other proximate factors (e.g., differential prey availability).

**Key words:** clutch size, environmental conditions, long-term sperm storage, phylogenetic inertia, reproductive cycles, spermatogenesis

## INTRODUCTION

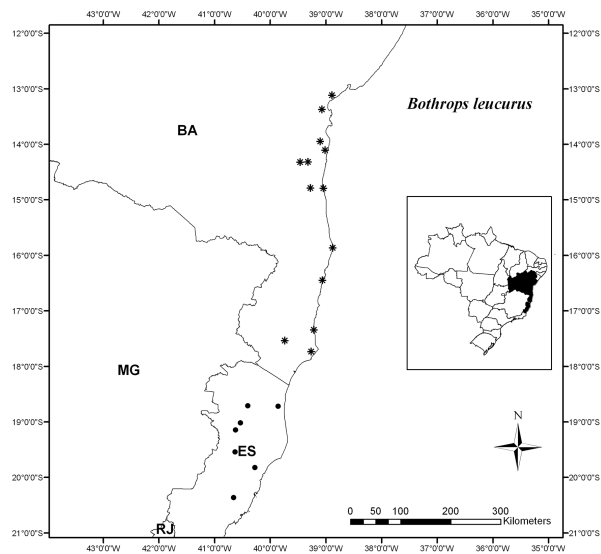
Rainfall seasonality is an important factor that influences the timing of reproduction. In oviparous snakes from tropical regions, it may favour seasonal reproduction due to the lack of periods with soil conditions capable of sustaining optimal embryogenesis (Brown & Shine, 2006). Although viviparous snakes are able to modulate conditions of embryo development through thermoregulation (Blackburn & Stewart, 2011), rainfall seasonality may also play a role in their timing of reproduction as it may affect prey availability and the activity patterns of predators.

*Bothrops leucurus* is a suitable model to test the hypothesis that rainfall seasonality influences the reproductive strategies of snakes. Although high temperatures are maintained throughout the year across the species' range, rainfall seasonality differs widely. *Bothrops leucurus* inhabits the Atlantic coastal forests of Brazil in the states of Espírito Santo, Bahia, Sergipe, Alagoas, Pernambuco, Ceará and Maranhão (Porto & Teixeira, 1995; Campbell & Lamar, 2004; Lira-da-Silva et al., 2009). This species may also occur in urban and disturbed areas such as plantations and cattle pastures (Ripa, 1997; Lira-da-Silva et al., 2009). Rainfall may be abundant throughout the year

(aseasonal, AS) or during spring and summer (seasonal, SE) throughout the geographical range of this species. Constant climatic conditions such as high temperatures and rainfall throughout the year are presumed ideal to maintain high prey availability, favourable conditions for spermatogenesis and sexual activity at any time of the year (Saint-Girons, 1982). We here compared the reproductive strategies of two populations of *B. leucurus* from two Brazilian states (Espírito Santo and Bahia) with different rainfall seasonality patterns. This is the first comprehensive study of reproductive strategies of *B. leucurus*, a snake species that is important to public health in Brazil due to the high incidence of envenomation caused by snakebites (Mise et al., 2007; Lira-da-Silva et al., 2009). Male to male combat and mating have been observed during autumn (Almeida-Santos & Salomão, 2002), and births that occurred from December (the beginning of summer) to April (the beginning of autumn) were recorded in captivity (Lira-da-Silva et al., 1994; Sant'Anna et al., 2001).

## MATERIALS AND METHODS

We examined a total of 336 specimens of *B. leucurus* (78 females and 90 males from Espírito Santo, ES; 32 females and 59 males from Bahia, BA; 12 immature



**Fig. 1.** Geographical origin of *Bothrops leucurus* specimens sampled. Circles represent municipalities from Espírito Santo (seasonal environment) and asterisks represent municipalities from Bahia (aseasonal environment).

individuals from ES and 6 from BA; 15 newborns from ES and 44 from BA) held in the following collections: Museu de Zoologia da Universidade Estadual de Santa Cruz (MZUESC), Museu de Ciência e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCT/ PUC-RS), Coleção Zoológica Gregório Bondar (CZGB) and Coleção Herpetológica Alphonse Richard Hoge at Instituto Butantan (IB). Individuals collected in ES originated from the municipalities of Colatina, São Domingos do Norte, São Gabriel da Palha, Nova Venécia, São Mateus, Domingos Martins and Aracruz (18°42'–20°21' S; 40°24'–40°39' W), from the Atlantic Forest and from disturbed areas. Specimens collected in BA originated from the municipalities of Ilhéus, Una, Caravelas, Teixeira de Freitas, Camamu, Valença, Belmonte, Itabuna, Aurelino Leal, Gongogi, Porto Seguro, Marau, Jaguaripe and Prado (13°6'–17°43' S; 38°53'–39°15' W), from the Atlantic Forest, and from plantations and disturbed areas (Fig. 1). We excluded any specimens that were previously maintained in captivity.

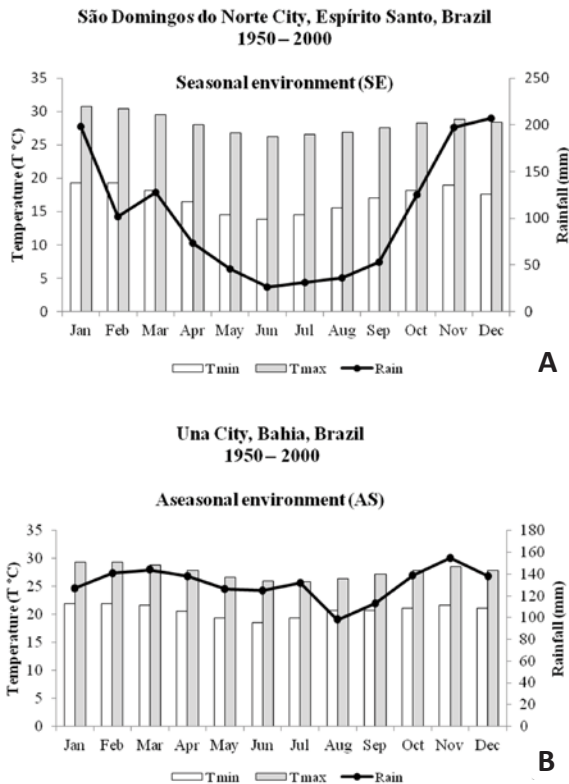
Climatograms were constructed for every municipality sampled using DIVA-GIS (version 7.1.7) and the WorldClim

database (current climate, time range from 1950–2000, v.1.3, 10 minute resolution). High temperatures are maintained throughout the year in all of the sampled municipalities at different patterns of rainfall. Dry and wet seasons are well defined in all localities from ES (seasonal environment, SE, Fig. 2A), while rainfall is abundant throughout the year in all municipalities sampled in BA (aseasonal environment, AS, Fig. 2B). In ES, the coldest months were June and July (with minimum temperatures ranging from 12.2–17.5°C), and the warmest month was February (with maximum temperatures ranging from 28.2–31.7°C). Annual precipitation varies between 1110 mm and 1289 mm among the sampled municipalities in ES. In BA, the coldest months were June and July (with minimum temperatures ranging from 18.0–19.5°C), and the warmest months were January and February (with maximum temperatures ranging from 28.2–30.7°C). Annual precipitation varies between 1400 mm and 2218 mm among the sampled municipalities in BA. We characterise seasons as summer (late-December to late-March), autumn (late-March to late-June), winter (late-June to late-September) and spring (late-September to late-December) to allow for comparisons with other studies.

We measured snout-vent length (SVL) to the nearest mm, and recorded the following data for females after dissection: (i) diameter of the largest ovarian follicle or the presence of embryos in females; (ii) presence/absence of uterine muscular twisting (UMT) in female uteri described as a sperm storage site in snakes (Almeida-Santos & Salomão, 1997; 2002; Barros et al., 2012); and (iii) presence of corpora lutea in the ovaries. For males, we recorded (i) length, width and thickness of the testes in males to determine testicular volume (TV) using the ellipsoid formula  $TV = 4/3 \cdot \pi \cdot a \cdot b \cdot c$ , where a=half of the length, b=half of the width and c=half of the thickness (Pleguezuelos & Feriche, 1999); and (ii) aspect (straight, opaque, or convoluted) and width of the ductus deferens close to the cloaca. These measurements were recorded to the nearest 0.1 mm. Some males presented multipartite testes (Werner & Drook, 1967). For these individuals, we measured each unit of the testes individually and the sum of all units was the final volume of the testes.

**Table 1.** Stages of the spermatogenic cycle in *Bothrops leucurus*. Adapted from Goldberg & Parker (1975) and Rojas et al. (2013).

Stages	Spermatogenic condition	Seasons
I	Complete regression: only spermatogonia and Sertoli cells	Winter
II	Early recrudescence: division of spermatogonia and primary spermatocytes, remnant spermatozoa in the lumen	Spring
III	Late recrudescence: primary spermatocytes and spermatids	Spring
IV	Early spermiogenesis: spermatids in metamorphosis	Summer
V	Spermiogenesis: mature spermatozoa in the lumen	Summer/Autumn
VI	Early regression: decrease of the seminiferous epithelium	Winter



**Fig. 2.** Climatic pattern in Espírito Santo (seasonal environment) and Bahia (aseasonal environment).

The presence of spermatozoa in the testes or ductus deferens was used as the main criterion to determine sexual maturity in males. The convoluted aspect of the ductus deferens was used as an additional criterion. Females were considered mature if they had follicles in secondary vitellogenesis, oviductal embryos or the presence of corpora lutea in the ovaries. Individuals who did not present any of these characteristics were considered immature.

The relation between female SVL and number of embryos was tested by linear regression. A student’s *t*-test was applied to test sexual dimorphism in SVL in mature individuals (differences between male and female SVL at each site) as well as differences in clutch size and newborn SVL between BA and ES. Differences between male and female SVL among populations were evaluated using Mann-Whitney tests. Analysis of covariance (ANCOVA) was employed to compare testes volume among the seasons, and SVL was used as the covariate. Differences of ductus deferens width among seasons were investigated using ANOVA.

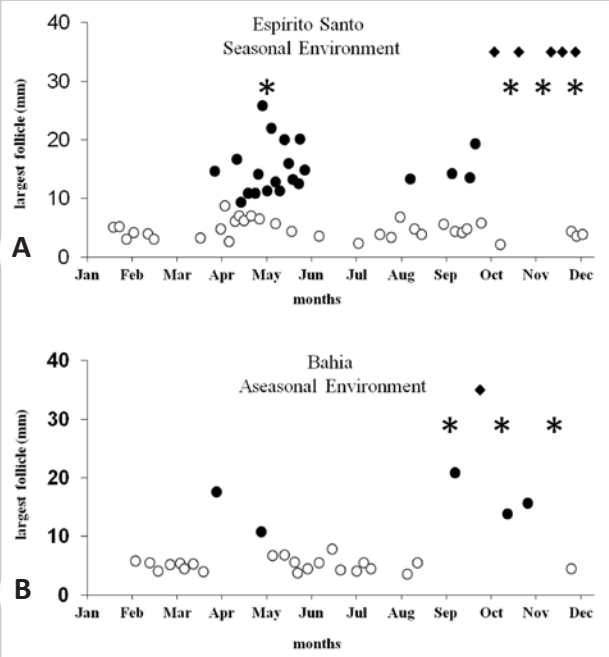
We collected the UMT of females (*n*=4 per population), as well as testes and ductus deferens of males (*n*=16 per population) for histological analysis. All organs were preserved in 70% alcohol and processed for light microscopy by the paraffin method. Sections were cut 5 µm thick, and stained for histology with hematoxylin and eosin (H/E). Slides were inspected under an Olympus BX51 microscope, morphometric measurements and images were obtained through the Image – Pro Express Olympus program. Morphological and structural variations in the seminiferous tubules were used to characterise the spermatogenic cycle. Structural changes

in the testes were registered by seasonal changes in the diameter of the seminiferous tubule and the height of the seminiferous epithelium and tested by Kruskal-Wallis (post-hoc test: comparisons by the Dunn method). We followed Goldberg & Parker (1975) and Rojas et al. (2013) in delineating spermatogenic stages in snakes (Table 1).

Statistical analyses were performed using Biostat (v.5.00), assuming *p*<0.05 as the criterion for significance. The analyses were carried out according to Zar (1999) and all variables were tested for normality and homoscedasticity prior to analysis. Non parametric tests were used in the absence of normal distribution or when data presented unequal variance. Graphs were made using GraphPad PRISM (v.5.04), and a map was made using ArcGIS (v.9.3).

RESULTS

Males from BA (*n*=4) and ES (*n*=10) below 47 cm SVL were considered immature. Analyses of gross morphology and histology confirmed the immature condition in females from BA (*n*=2, 44–49 cm SVL) and from ES (*n*=2, 46–53 cm SVL). Males from BA (87.9±17.13 cm SVL, *n*=59, range 53–127 cm) were larger than males from ES (76.6±12.06 cm SVL, *n*=90, range 52–103.5 cm, *Z*=4.23, *p*<0.01), and females from BA (104.08±14.73 cm SVL, *n*=32, range 69.3–136 cm) were larger than females from ES (76.6±10.93 cm SVL, *n*=78, range 55.5–101.5 cm, *Z*=6.34, *p*<0.01). No significant difference of SVL was observed between the sexes in ES (*t*=0.0878, *p*=0.47), but females were larger than males in BA (*t*=-4.2346, *p*<0.01).



**Fig. 3.** The female reproductive cycle of *Bothrops leucurus* from (A) Espírito Santo and (B) Bahia. Seasonal variation in the diameter of the largest ovarian follicle and the occurrence of embryos in mature females of *Bothrops leucurus*. Open circles: primary vitellogenic follicles; solid circles: secondary vitellogenic follicles; asterisks: early embryos in the oviducts (only yolk visible); diamonds: late embryos in the oviducts.

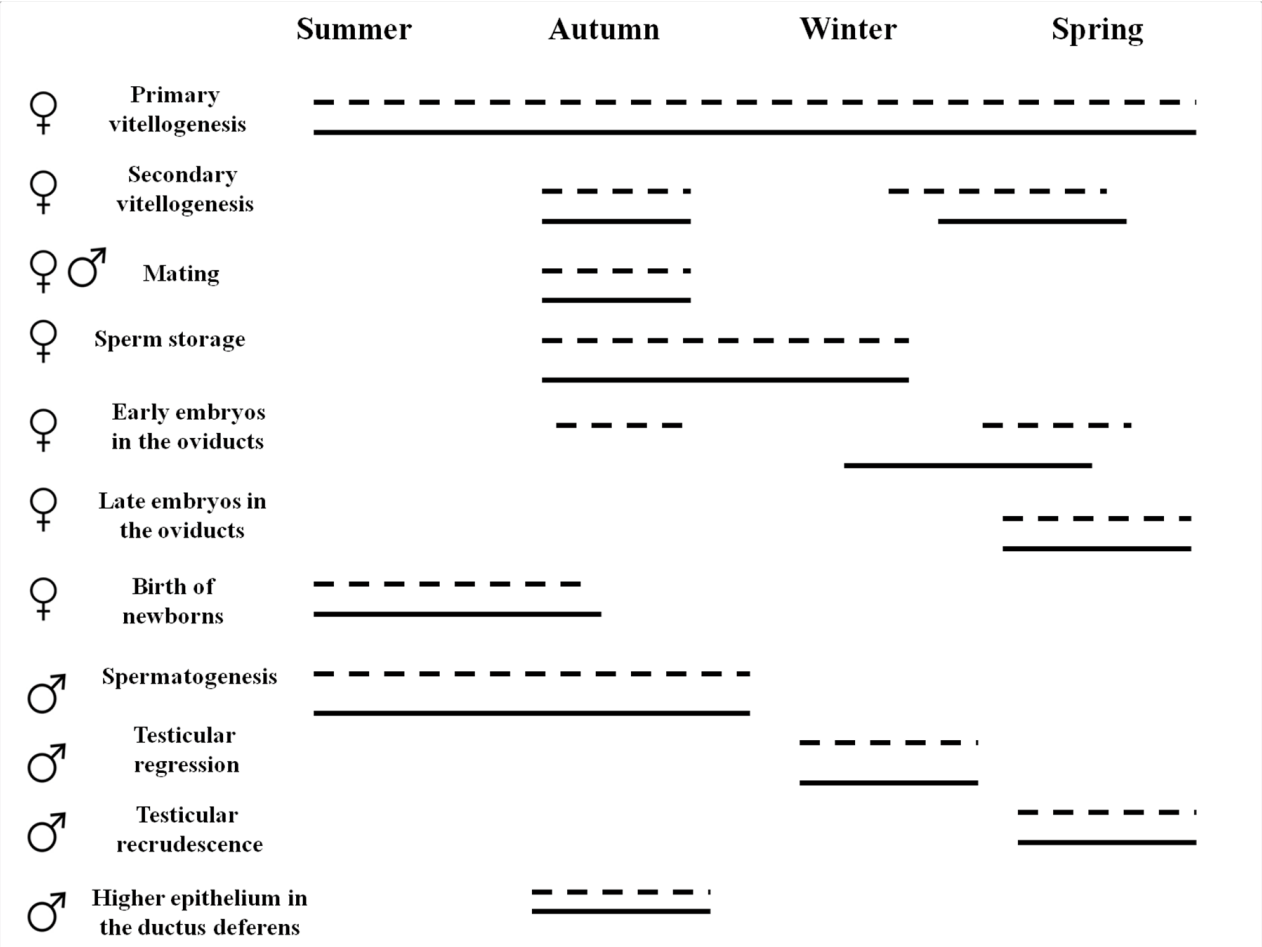


Fig. 4. Reproductive events in *Bothrops leucurus* from Bahia (continuous line) and Espírito Santo (dotted line).

Females exhibiting primary follicles were present throughout the year in both populations. Females with secondary vitellogenic follicles were collected from March to November. A single female from Nova Venécia (ES) exhibited recently ovulated eggs in the oviducts in May. Fully developed embryos were found in the oviducts from October to January (spring/summer) in females from ES and in October (spring) in a female from BA (Figs. 3, 4).

Clutch size was larger in BA ( $16.7 \pm 8.04$  newborns,  $n=6$ ) than in ES ( $7.6 \pm 3.13$  newborns,  $n=5$ ,  $t=2.36$ ,  $p=0.04$ ), and positively correlated with female SVL ( $r^2=0.47$ ,  $p<0.01$ ). Newborns from BA were larger ( $26.56 \pm 3.01$  cm SVL,  $n=44$ , range 20.8–32.5 cm) than newborns from ES ( $24.45 \pm 4.26$  cm SVL,  $n=15$ , range 18–31 cm,  $t=2.10$ ,  $p=0.04$ ).

Uterine muscular twisting (UMT) was macroscopically observed in seven females from BA exhibiting primary ( $n=4$ ) and secondary ( $n=3$ ) vitellogenic follicles in the ovaries between March and November. In ES, UMT occurring synchronously with secondary vitellogenesis was observed most frequently in autumn (May,  $n=5$ ) and late-winter (September,  $n=1$ ), as well as in 9 females from ES with follicles in primary vitellogenesis from January to September. Histological analysis of the UMT showed the presence of spermatozoa in one female from Bahia; this female exhibited quiescent ovarian follicles (primary vitellogenesis) in autumn (Fig. 5). Microscopically, the UMT in the posterior (non-glandular) uterus was

characterised by deep longitudinal furrows with mucosal folds where spermatozoa are stored. This sperm storage region is characterised by a pseudostratified columnar epithelium composed of interspersed ciliated and secretory cells (Fig. 5).

Timing of spermatogenesis does not differ between ES and BA populations (Fig. 4). The spermatogenic cycle is highly seasonal. Spermiogenesis was only observed

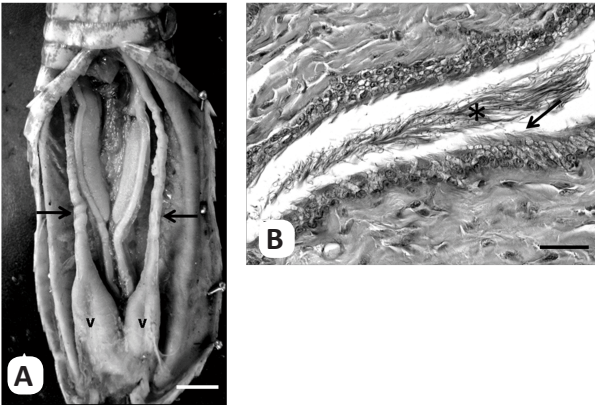


Fig. 5. Uterine muscular twisting (UMT) in *Bothrops leucurus*. (A) Macroscopical view showing the vagina (v) and the posterior (non-glandular) uterus. Arrows indicate the UMT in the uterus. Bar=10 mm. (B) Microscopical view. The arrow indicates the ciliated epithelium and the asterisk indicates the presence of spermatozoa. Bar=30  $\mu$ m.



**Table 2.** Structural variation of the testes of *B. leucurus* between different seasons in Bahia (BA) and Espírito Santo (ES) populations. Post hoc analysis: significant differences ( $p<0.05$ ) between seasons are indicated by superscript letters. For example, summer is represented by letter A and seminiferous tubule diameter in BA is different (larger) in summer (A) from winter (C) and spring (D).

Variables	Seminiferous tubule diameter (µm)		Seminiferous epithelial height (µm)	
Season	BA	ES	BA	ES
Summer <sup>(A)</sup>	290.63±60.83 <sup>C,D</sup>	264.92±47.11 <sup>C</sup>	65.41±20.65 <sup>C</sup>	90.98±16.40 <sup>C,D</sup>
Autumn <sup>(B)</sup>	311.68±59.30 <sup>C,D</sup>	302.29±51.73 <sup>C,D</sup>	94.36±40.52 <sup>C,D</sup>	81.24±41.97 <sup>C,D</sup>
Winter <sup>(C)</sup>	166.47±18.87 <sup>A,B</sup>	180.19±23.37 <sup>A,B,D</sup>	30.55±7.16 <sup>A,B,D</sup>	33.11±6.18 <sup>A,B</sup>
Spring <sup>(D)</sup>	194.11±38.05 <sup>A,B</sup>	232.24±22.94 <sup>B,C</sup>	57.79±34.55 <sup>B,C</sup>	34.07±7.48 <sup>A,B</sup>

during summer and autumn when seminiferous epithelium height (SEH) and seminiferous tubules diameter (STD) increased in both populations (Tables 1, 2, Figs. 4, 6, 7). Microscopic measurements of the testes peaked at the time of spermiogenesis in both populations, and these parameters showed a similar pattern in BA (SEH,  $H=34.1677$ ,  $p<0.01$ ; STD,  $H=57.1169$ ,  $p<0.01$ ) and ES (SEH,  $H=60.1362$ ,  $p<0.01$ ; STD,  $H=56.2298$ ,  $p<0.01$ ; Table 2, Fig. 7). Considering testes volume as a macroscopic indicator of spermatogenesis, this parameter was positively correlated with SVL in males from ES ( $F=37.0248$ ,  $r^2=0.2961$ ,  $p<0.01$ ) and BA ( $F=53.7037$ ,  $r^2=0.4851$ ,  $p<0.01$ ). However, no significant differences occurred among the seasons in ES ( $F=1.6464$ ,  $p=0.18$ ) and BA ( $F=0.7083$ ,  $p=0.55$ ), although a slight increase in testes volume was observed in summer and autumn for the BA population (Fig. 8A).

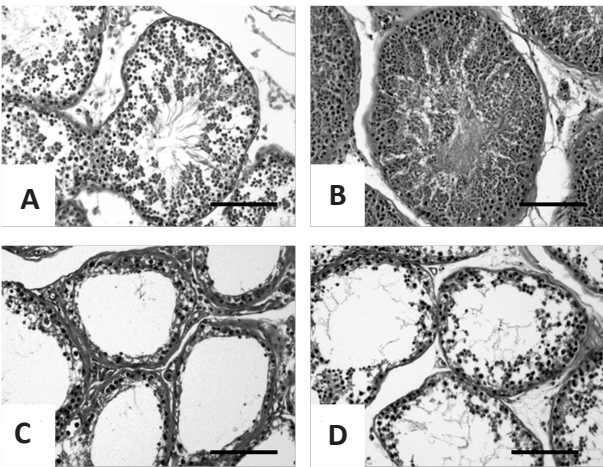
The width of the ductus deferens did not differ among seasons for either population (BA,  $F=0.9944$ ,  $p=0.59$ ; and ES,  $F=1.1742$ ,  $p=0.32$ , Fig. 8B). Microscopically, two conditions were detected in the ductus deferens of both populations of *B. leucurus*: (i) low and pseudostratified epithelium surrounded by a muscular layer and an external serosa with a spermatozoa mass in the lumen (observed throughout the year, Fig. 9A), and (ii) the presence of a higher epithelium with irregular projections

and the formation of crypts (characteristics of an ampulla ductus deferentis, Sever, 2004; Trauth & Sever, 2011; Rojas et al., 2013) with a spermatozoa mass in the lumen (observed only during the autumn, Fig. 9B). We did not observe a relationship between the macroscopic aspect of the ductus deferens (straight, opaque or convoluted) and the microscopic conformations of the epithelium.

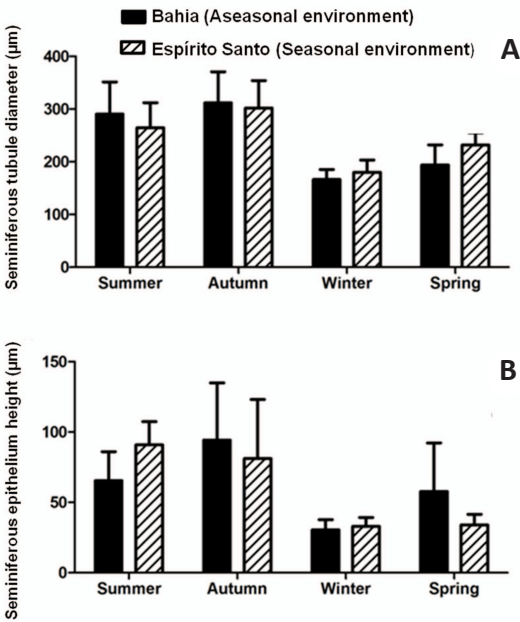
DISCUSSION

Female reproductive cycle

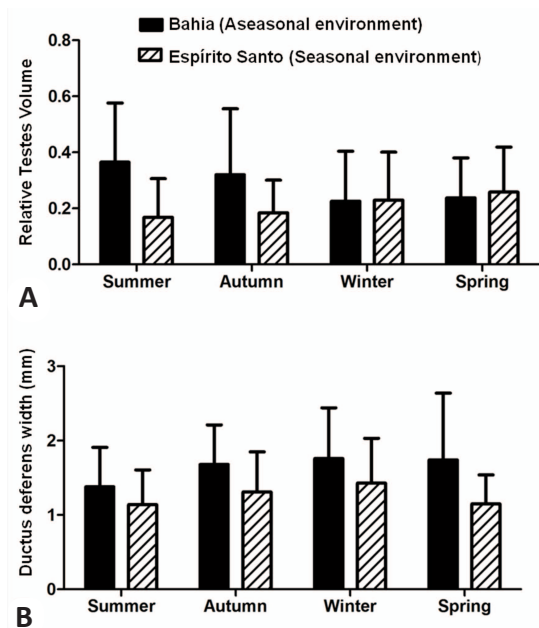
Rainfall seasonality does not seem to exert any influence on *B. leucurus* reproductive events, but it may have a role on traits such as adult size (SVL), clutch size and size of newborns. Spermatozoa were found in the female reproductive tract in autumn, which confirms that autumn is the mating season in the wild. Ovulation could occur during autumn because high temperatures are maintained throughout the year and it likely allows adequate embryonic development to occur at any time of the year (Vinegar, 1974; Yan et al., 2011) in the geographical range of *B. leucurus*. However, ovulation



**Fig. 6.** Sections of the testes in *Bothrops leucurus*. (A) Spermatogenesis during summer (stage 5). (B) Maximum height of the seminiferous epithelium during autumn (stage 5). (C) Total regression during winter (stage 1). (D) Early recrudescence (stage 2) during spring. Bars=100 µm.



**Fig. 7.** Seasonal variation of microscopic parameters of the spermatogenesis in *Bothrops leucurus* from Espírito Santo and Bahia. (A) Seminiferous tubule diameter (µm) (STD). (B) Seminiferous epithelium height (SEH, µm).



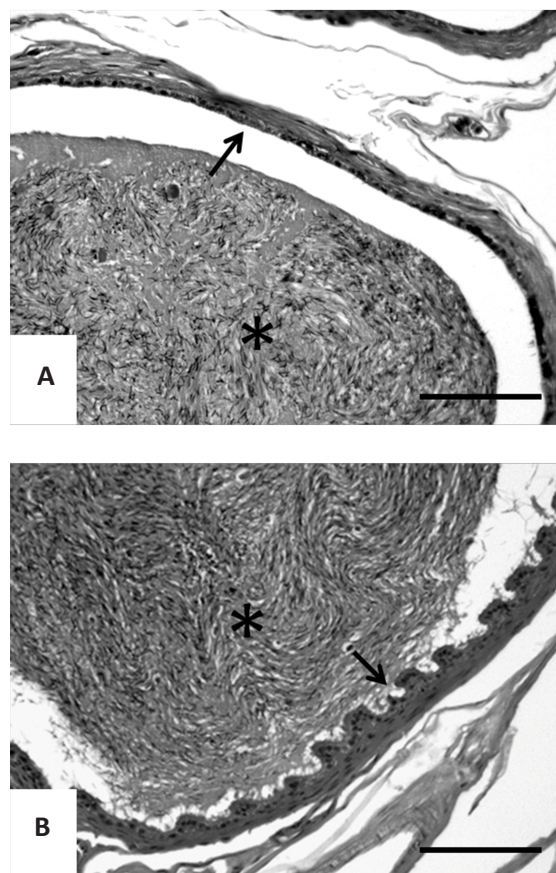
**Fig. 8.** Seasonal variation of macroscopic parameters of the male reproductive cycle in *Bothrops leucurus* from Espírito Santo and Bahia. (A) Relative testes volume (indicator of spermatogenesis). (B) Ductus deferens width (indicator of male sperm storage).

and mating did not occur synchronously in females from BA and most females from ES. Births were observed only during the summer in both populations (Almeida-Santos & Salomão, 2002; Sant'Anna et al., 2001), regardless of ovulation timing. Therefore, sperm storage must be an obligatory component of the female reproductive cycle to guarantee the occurrence of fertilisation and successful reproduction. Two regions in the oviducts of female snakes are recognised as sperm storage sites: (i) the posterior (non-glandular) uterus, where UMT occurs; and (ii) the posterior infundibulum where some specialised structures (seminal receptacles) have been described for other snake species (Siegel et al., 2011). Marinho et al. (2008) observed an increase in peptidase activities in the presence of UMT associated with the reduction of spermatozoa motility and the maintenance of fertilisation capacity, features that are likely related to adaptations of sperm storage. However, more research, including DNA tests of the sperm maintained in the UMT, is necessary to determine a possible role of this structure in sperm competition (Booth & Schuett, 2011). In this study, we only analysed one sperm storage site, the UMT, but future studies should investigate the occurrence of sperm storage in infundibular glands in *B. leucurus* for a better understanding of sperm storage strategies in this species.

Female reproductive cycle of *B. leucurus* may be considered as seasonal semi-synchronous (Mathies, 2011) because some variability in timing of some reproductive events (e.g., ovulation, vitellogenesis) is observed among individuals, but timing of parturition does not vary. Therefore, phylogenetic inertia seems to exert a determinant role on the timing of reproduction in *B. leucurus* (Almeida-Santos & Salomão, 2002). However, environmental variables may have some influence on the

reproductive patterns of the species of the *B. atrox* group. Ovulation occurred in July in one female of *B. moojeni* from the Brazilian Cerrado (Nogueira et al., 2003) and in May in one *B. leucurus* female from ES (this study). A female of *B. atrox* from French Guiana exhibited well developed embryos in her oviducts in July (Gasc & Rodrigues, 1980), indicating that these species may have potential plasticity in the timing of parturition. Two populations of *B. asper* isolated by mountains in Costa Rica also exhibit interspecific variation in some reproductive events (e.g., vitellogenesis and births), which is attributed to different climatic conditions between the two regions (Solórzano & Cerdas, 1989).

Available data on the occurrence of oviductal embryos and parturition in captivity in ES and BA populations indicate that births most likely occur in the same period in both populations, similar to other species of the genus *Bothrops* (December to April, summer to early-autumn) (Lira-da-Silva et al., 1994; Almeida-Santos & Salomão, 2002; Sant'Anna et al., 2001). A previous study on *B. leucurus* from BA showed similar results for the size of newborns (Lira-da-Silva et al., 1994). Clutch size is related to the female SVL in *B. leucurus* and several squamate species (e.g. Scartozzoni, 2009; Mesquita et al., 2013; Panzera & Maneyro, 2013). Rainfall seasonality would indirectly influence this pattern because females grow larger in BA municipalities where rainfall is abundant



**Fig. 9.** Ductus deferens in *Bothrops leucurus*. Asterisks indicate the mass of spermatozoa. Arrows indicate the epithelium. (A) Distal ductus deferens presenting low and pseudostratified epithelium. (B) Presence of folds and crypts in the epithelium. Bars=100 µm.

throughout the year, which should ensure abundant food resources and theoretically optimal conditions for growth in this species, whereas in ES, there might be a decrease in prey availability during the dry season, which may be responsible for the smaller size of individuals in this population. Ontogenetic diet shift (from ectothermic to endothermic prey) occurs in *B. leucurus* (Martins et al., 2002). Therefore, juveniles from an aseasonal environment (with constant rain) as BA could grow faster and longer due to higher availability of some prey (e.g., amphibians) in comparison to conspecific individuals from ES which has a dry season with probable low availability of ectothermic prey (amphibians and squamates). This hypothesis may be tested by an evaluation of prey availability in both areas (cf. Brown & Shine, 2006). Environmental factors have some influence on the variation in clutch size and other reproductive traits in four populations of the Chinese cobra (*Naja atra*) (Ji & Wang, 2005). Experiments conducted in captivity by Ford & Seigel (1989) and Seigel & Ford (1991) with *Thamnophis marcianus* and *Elaphe guttata* showed that females that consumed more caloric diets produced larger clutches.

### Sexual maturation and dimorphism

Early maturation is characteristic for *B. leucurus*, although many other viperid snakes exhibit late maturation (Saint-Girons, 1957). Males of *B. leucurus* from BA and ES attain sexual maturity at similar sizes. Females from ES also mature at a similar size compared to conspecific males. Unfortunately, it was not possible to sample females from BA who had a 50–65 cm SVL to provide a comprehensive analysis of intraspecific variation in sexual maturation in *B. leucurus*.

The observed patterns of sexual dimorphism in *B. leucurus* are unexpected for a species that employs male-to-male combat as a reproductive strategy (Almeida-Santos & Salomão, 2002). Generally, males are larger than females in species that exhibit male-to-male combat behaviour (Shine, 1994), but for the species of the *B. atrox* group (*B. moojeni*, *B. leucurus*, *B. atrox*), females may exhibit similar sizes or be larger than conspecific males (Almeida-Santos & Salomão, 2002, this study). Females are larger than males in other species of the genus *Bothrops* (Hartmann et al., 2004; Almeida-Santos, 2005; Monteiro et al., 2006; Barros, 2011). Thus, the sexual dimorphism pattern observed in *B. leucurus* may be influenced by phylogenetic inertia.

### Male reproductive cycle

Most studies on the reproductive biology of Neotropical snakes that include parameters of the male reproductive cycle (e.g., Pizzatto & Marques, 2002; Marques et al., 2006; Scartozzoni et al., 2009; Pinto et al., 2010; Mesquita et al., 2013) rely on testes volume or length to infer timing of spermatogenesis and are based on the premise that testes are larger during sperm production (as demonstrated in *Vipera berus* by Volsøe, 1944). However, conclusions based on this parameter may be misleading without histological evidence (Mathies, 2011; Rojas et al., 2013). On the other hand, microscopic

measurements of the testes (STD and SEH) were very good indicators for the timing of spermatogenesis, which occurs in autumn synchronously to increased secretory activity in the ductus deferens (presence of an ampulla ductus deferentis) and mating in both populations. Males of *Crotalus durissus* from the caatinga in north-eastern Brazil, a region with high temperatures throughout the year and pronounced dry and wet seasons, also exhibit spermatogenic activity in the testes at the time of mating (Barros et al., 2012). Sperm production, however, does not occur synchronously during the mating season in *C. durissus* and *B. jararaca* from south-eastern Brazil, a region with a colder climate and pronounced rainfall seasonality (Salomão & Almeida-Santos, 2002; Almeida-Santos, 2005; Barros et al., 2012). Therefore, temperature seems to have more of an influence on the timing of spermatogenesis for snake species than rainfall seasonality. The maintenance of high temperatures throughout the year in the geographical range of *B. leucurus* allows spermatogenesis to occur at any time of the year, including the mating season. This may not be a possibility for snakes that live in colder climates and that need to store sperm in the ductus deferens until mating, which adds an energetic cost to the reproductive event (Barros et al., 2012).

The width of the ductus deferens is considered a good indicator of male sperm storage in snakes (e.g., Almeida-Santos et al., 2006), but we failed to find clear evidence of male sperm storage in populations of *B. leucurus* in BA and ES. The ampulla ductus deferentis, which was observed in *B. leucurus*, has already been described for other Squamata species (Sever, 2004; Siegel et al., 2009; Trauth & Sever, 2011; Rojas et al., 2013). Its functions include sperm storage, secretory activity and phagocytosis (Akbarsha & Meeran, 1995; Akbarsha et al., 2005). In *B. leucurus*, an ampulla ductus deferentis was observed only during the mating season (autumn) when spermatogenesis peaked. It reinforces the possibility of some function involving sperm storage and/or secretory activity for sperm nutrition of the ductus deferens in this snake species. Sperm was observed throughout the year in the ductus deferens, in the presence and absence of the ampulla. Future studies should investigate if sperm viability and surveillance differ between the seasons and/or the ductus conformation.

Reproductive traits may exhibit intraspecific variation in snake species (Santos et al., 2005; Ji & Wang, 2005; Barros et al., 2012), including variation on timing of male (e.g., spermatogenesis/testicular regression, Barros et al., 2012) and female (e.g., vitellogenesis and egg-laying, Pizzatto et al., 2008) reproductive cycles. On the other hand, phylogenetic inertia may have a major role in determining the timing of reproductive events in some squamates (James & Shine, 1985, 1988; Almeida-Santos & Salomão, 2002; Barros et al., 2012), as observed in *B. leucurus* reproductive cycles. The possible influence of environmental conditions on other reproductive traits of *B. leucurus* (e.g., clutch size) deserves a more comprehensive investigation in the future, including larger sample sizes for analysis and more tests on the hypotheses in the field and captivity. Females of different



sites may be maintained in captivity receiving similar diets to test the influence of prey availability in clutch size (Ford & Seigel, 1989). Newborns of a clutch of *B. leucurus* could be divided in two groups which should be raised with different diets (high availability of prey throughout the year versus low prey availability during the dry season) to verify if this factor may be responsible for differences in SVL observed between populations in this study. A seasonal evaluation of *B. leucurus* prey availability and consumption in both sites should also clarify this hypothesis.

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