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Abstract.—The Golden Lancehead (*Bothrops insularis*) is a critically endangered snake endemic to the Queimada Grande Island in southeastern Brazil. Here, we provide data on reproductive biology of *B. insularis* obtained in the field and from preserved museum specimens and compare our results with its mainland relative *Bothrops jararaca*. Similar to other *Bothrops* species, females of *B. insularis* attain larger body sizes than males, but the sexual size dimorphism (SSD) value is much lower than in *B. jararaca*. The seasonal timing of reproduction of *B. insularis* is similar to mainland *B. jararaca* as well as other species of the genus *Bothrops*. Courtship observed in nature takes place in autumn and early winter (March to July); vitellogenesis occurs from summer to spring (March to December); ovulation and fertilization occur in early spring (September); embryonic development takes place in middle spring and summer (October to April); and recruitment of newborns is restricted to the summer (February and March). Both offspring size and offspring mass of *B. insularis* are similar to that observed in *B. jararaca*, but *B. insularis* produces a high frequency of atretic follicles. Apparently *B. insularis* has a lower reproductive frequency than *B. jararaca*, which is highly variable from year to year. The distinct reproductive traits of *B. insularis* (compared to *B. jararaca*) seem to be related to the ecological conditions of its insular environment, which may eventually be considered for the conservation management of this threatened species.

The Neotropical pitvipers of the genus *Bothrops* comprise about 40 species (Campbell and Lamar, 2004). These snakes are distributed widely throughout South America in a great variety of habitats (Martins et al., 2001; Campbell and Lamar, 2004). Available data indicate that the genus *Bothrops* (sensu lato, but see Fenwick et al., 2009) is conservative in some ecological traits (e.g., Martins et al., 2002). For example, most species are diet generalists, although five species are diet specialists including the two insular endemic species (Martins et al., 2002). Certain aspects of reproduction, such as reproductive cycles, seem to be similarly conservative (Almeida-Santos and Salomão, 2002).

However other aspects, such as fecundity, may be variable because of the relationship of fecundity to body size, which differs among the species of *Bothrops* (Martins et al., 2001). Moreover, fecundity may be influenced by resource availability in different regions or habitats (Blem 1982; Seigel and Fitch, 1985; Seigel and Ford, 2001; Shine, 2005).

Two endemic species of the genus *Bothrops* (*Bothrops alcatraz* and *Bothrops insularis*) inhabit small islands from southeastern Brazil (Marques et al., 2002a,b). These island species are phylogenetically related to mainland *Bothrops jararaca* (Marques et al., 2002a,b; Graziotin, 2006; Fenwick et al., 2009). The island species differ mainly from mainland *B. jararaca* by their smaller body size and diet specializations (Marques et al., 2002a,b, 2012; Martins et al., 2002). Thus, the ecological features of the island species and particular insular environmental conditions provide an interesting scenario for the study of reproduction. *Bothrops alcatraz* is not common in nature, and herpetological collections house few preserved specimens (Marques et al., 2002a). However, *B. insularis* is abundant in nature; this species has been collected intensively at Queimada Grande Island between 1914 and 1970, and field trips throughout the last 15 yr have allowed us to gather data on reproduction. Quadrat plot studies in this small island reveal a population of approximately 2,100 snakes, and encounter rate data obtained in the last 12 yr suggested that the density of this snake decreased drastically in this period (Martins et al., 2008).

The Golden Lancehead is included as critically endangered in the Brazilian List of Endangered Animals (Machado et al., 2005); consequently, data on reproductive traits are essential for conservation. The aim of this study is to describe the reproductive biology of *B. insularis* and address the following questions: Is the sexual dimorphism of *B. insularis* similar to other related *Bothrops*? Is the reproductive cycle of *B. insularis* similar to that observed in other *Bothrops*? Does the fecundity of insular *B. insularis* differ from that of mainland *B. jararaca*? Does the fecundity of *B. insularis* vary annually?

Material and Methods

Study Species and Area.—Queimada Grande (hereafter QGI) is a small island (0.43 km$^2$) located approximately 34 km off the coast of São Paulo, southeastern Brazil (24°30′S and 43°42′W) (Amaral, 1921a; Duarte et al., 1995; Martins et al., 2008) with altitudinal range 0–200 m above sea level. Monthly mean air temperatures range from 18.3°C in August to 27.2°C in March, and rainfall ranges from 0.2 mm in July to 135.2 mm in December (Fig. 1). The climate in QGI is similar to the adjacent mainland (Nimer, 1989). About 0.25 km$^2$ of the island is covered by rain forest (Lower Montane Rain Forest, cf. Oliveira-Filho and Fontes, 2000). The remaining areas are bare rock and open areas covered mostly with grasses; many of these open areas result from deforestation by humans (Marques et al., 2002b). The forest is the main habitat of *B. insularis*, which is rarely found in grassy areas (Martins et al., 2008). The snake fauna also includes the slug-eater *Dipsas albifrons*. Migratory passerine birds visit the island periodically and provide the main food for *B. insularis* (Marques et al., 2002b; 2012, Macarrro, 2010).

Reproductive Data.—Preserved specimens (*N* = 409; 246 females and 163 males) from the collection of the Instituto Butantan, collected at QGI between 1914 and 1970, were examined in this study. The following data were taken from each preserved specimen: (1) snout–vent length (SVL); (2) sex; (3) reproductive maturity or immaturity (females were considered mature if they had embryos in oviducts or vitellogenic follicles; males were considered mature if they had enlarged testes and opaque deferent ducts, Shine, 1980, 1982); and (4) diameter of the largest ovarian follicles and the number and length (SVL) of embryos. Degree of sexual size dimorphism (SSD) was 1– (mean adult...
SVL of the larger sex/mean adult SVL of the smaller sex) (see Shine 1994a). For comparative analysis of fecundity, we obtained data on SVL and number of vitellogenic follicles or embryos of mainland B. jararaca. Means are followed by 1 SD. A total of 36 preserved reproductive females of B. jararaca from southeastern Brazil were examined. Additionally, the reproductive status of females of B. insularis was determined in nature throughout 13 yr. Fieldwork was carried out during regular visits to QGI between 1995 and 2008. Excursions lasted 2–5 days and were planned to sample snakes during all seasons throughout the year. However, adverse weather conditions prevented sampling in all four seasons in certain years. At different times of the day, snakes were searched for along a north-south trail that crosses the island. At least four trained people searched for snakes visually, two searching the ground and two searching the vegetation. For each captured snake, we recorded biometrical data (snout-vent length, SVL; tail length, TL; body mass), sex, reproductive condition, stomach content, microhabitat use, and time of the day. The reproductive condition of females was evaluated by palpation of the abdomen (Fitch, 1987). This method enables us to detect presence of growing follicles or embryos. Occasional interactions between males and females were recorded by photography and/or videotapes during the fieldwork. The photos and videotape sequence are housed at Laboratório de Ecologia e Evolução of Instituto Butantan.

RESULTS

Body Size and Sexual Dimorphism.—Mature males averaged 619.4 ± 58.4 mm SVL (range = 505–776 mm, N = 153), and mature females averaged 721.2 ± 95.3 mm SVL (range = 555–1016 mm, N = 223). The difference in body size between sexes was highly significant (t = 11.79; df = 374; P < 0.001). The degree of sexual dimorphism (SSD) was 0.16.

Reproductive Cycles.—Females with ovarian follicles in vitellogenesis (≥ 10 mm) were found from March to December, except for two in February (Fig. 2). Ovulation occurred in early spring, and females with oviductal embryos were recorded from October to April. From October to January (spring–summer) the embryos were in early stages of development, and from April (autumn), these were fully developed (Fig. 2). The data from four captive gravid females are in agreement with those from dissected specimens, since parturitions were recorded in February (N = 1), March (N = 2), and April (N = 2). Forty-four (18%) of the 246 females of B. insularis were reproductively active (32 females had vitellogenic follicles and 6 females had embryos or eggs without visible embryos in the oviducts).

Courtship and Mating.—Eight records of interaction between males and females in nature were recorded (Table 1). In June 2007 (end autumn–early winter), four interactions between males and females were observed. In this time, the same number of males and females was found in the field (usually in a trip the females outnumbered males). Moreover, during this trip, the highest number of males in locomotion was recorded: a total of 25 males (40%) were crawling, whereas in all other trips, the percentage of active males was lower than 9%. One male was observed moving toward a female in a tree, apparently having followed the female’s scent trail. The female initially was climbing, then rested on a branch, and the male climbed toward her (Fig. 3). Unfortunately we lost sight of the snakes at this point. Another male was observed when it moved toward a female on the ground. After this episode, courtship was recorded. This courtship was observed over about 4 hr (and recorded by video and photograph, Fig. 3). The male inspected the anterior dorsum of the female, with intense tongue flicking and rubbing his “chin”
along her back. At the start of courtship, the female extended her forebody but did not raise the tail nor open the cloaca (cloaca “gaping”). The female remained with its posterior body coiled during the inspection by the male. Occasionally, the male presented erratic body movements. Copulation was not observed in nature. All females courted (N = 4) did not have vitellogenic follicles. Two copulations were recorded in captive snakes in late autumn and early winter (Table 1).

**Fecundity**—The number of vitellogenic follicles for *B. insularis* (mean 8.2 ± 4.2, range 3–20, N = 29) was less (Mann–Whitney U-test: U = 54.0, P < 0.001) than for *B. jararaca* (mean 19.6 ± 6.3, range = 11–36, N = 36). Additionally, *B. insularis* females exhibited a high incidence of atretic follicles (44.8%), whereas *B. jararaca* had none. Because litter size was correlated with maternal body size in both species (*B. insularis*: r = 0.55, N = 29, P = 0.002; *B. jararaca*: r = 0.38, N = 36, P = 0.02; Fig. 4), we reanalyzed the data to control for the effects of maternal SVL on litter size. After removing atretic follicles from the data, and confirming that litter sizes/maternal body size slopes were similar for each species (ANCOVA: F = 0.036, P = 0.849), differences in mean litter sizes were not significantly different (ANCOVA: F = 0.003, P = 0.953).

Specimens found in the field from June to December throughout the last 13 yr were classified as either reproductive (gravid, or vitellogenic follicles) or non-reproductive (without follicles detected by palpation). A comparison of the reproductive adult females found in different years showed that this proportion is highly variable from year to year, ranging from 0 to 0.5 (Fig. 5).

**Newborn Individuals.**—Preserved neonates born in captivity housed in the IB collection averaged 217 ± 24.3 mm SVL (range = 174–285 mm, N = 60). From 831 individuals found in nature between 1995 and 2007, only 5 (0.6%) showed body size of newborns (snakes <300 mm SVL). Mean body sizes of these newborns, found between March and May, were 265 ± 26.7 mm (range = 240–298 mm) SVL and 13.6 ± 5.7 g (range = 8–19 g) body mass.

**DISCUSSION**

*Bothrops insularis* females have a larger body size than males, like most other species of the genus (Sazima, 1992; Valdujo et al., 2002; Nogueira et al., 2003; Hartmann et al., 2004). This is the most common condition among snakes and is generally correlated with absence of male–male combat behavior (Shine, 1978, 1994a). There are no observations of fights in *B. insularis* throughout many years of studies although there are many records of interactions between males and females in nature.

**Table 1. Interactions between males and females of *Bothrops insularis* recorded in Queimada Grande Island or in captivity.**

<table>
<thead>
<tr>
<th>Observation</th>
<th>Substrate</th>
<th>Date</th>
<th>Duration of event</th>
<th>Observer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mating on tree and ground</td>
<td>tree and ground</td>
<td>Aug/Sep</td>
<td></td>
<td>Amaral (1921)</td>
</tr>
<tr>
<td>Courtship in the field</td>
<td>tree</td>
<td>Jul/95</td>
<td></td>
<td>M. Martins</td>
</tr>
<tr>
<td>Courtship in the field</td>
<td>ground</td>
<td>Mar/02</td>
<td></td>
<td>O. Marques and M. Martins</td>
</tr>
<tr>
<td>Courtship in captivity</td>
<td>ground</td>
<td>Jun/06</td>
<td>~ 40 min</td>
<td>S. Travaglia-Cardoso, pers. comm.</td>
</tr>
<tr>
<td>Courtship in captivity</td>
<td>ground</td>
<td>Jun/06</td>
<td>~ 15 min</td>
<td>S. Travaglia-Cardoso, pers. comm.</td>
</tr>
<tr>
<td>Courtship in the field (female with prey)</td>
<td>ground</td>
<td>Jun/06</td>
<td>~ 30 min</td>
<td>F. Barbo, pers. comm.</td>
</tr>
<tr>
<td>Courtship in the field (female with prey)</td>
<td>ground</td>
<td>Jun/07</td>
<td>~ 4 h</td>
<td>This work</td>
</tr>
<tr>
<td>Male searching female in the field</td>
<td>tree</td>
<td>Jun/07</td>
<td>~ 4 h</td>
<td>This work</td>
</tr>
<tr>
<td>Male and female coiling together</td>
<td>tree</td>
<td>Jun/07</td>
<td>~ 2.5 h</td>
<td>S. Travaglia-Cardoso, pers. comm.</td>
</tr>
<tr>
<td>Mating in captivity</td>
<td>ground</td>
<td>Jun/08</td>
<td>~ 3 h</td>
<td>S. Travaglia-Cardoso, pers. comm.</td>
</tr>
<tr>
<td>Courtship in the field (female with prey)</td>
<td>ground</td>
<td>Jul/08</td>
<td>~ 3 h</td>
<td>This work</td>
</tr>
</tbody>
</table>
indicating that male combat behavior does not occur in this species. However, the value of SSD in *B. insularis* (0.16) is similar to those found in the *Bothrops atrox* group (0.10–0.15), and combat behavior in the genus *Bothrops* may be restricted to this group (Almeida-Santos and Salomão, 2002; Hartmann et al., 2004). Moreover, the value of SSD of *B. insularis* differs highly from that of *B. jararaca* (0.50), which does not exhibit combat, even though these two species are phylogenetically close (Graziotin et al., 2006; Fenwick et al., 2009). The higher value of SSD in *B. jararaca* is attributable to the fact that its females attain a much larger size (up to 1,500 mm SVL), which may be related to an increase in fecundity (Almeida-Santos, 2005). The females of *B. insularis* rarely reach up to 1,000 mm SVL (Amaral, 1921a; Guimarães et al., 2010). *Bothrops jararaca* usually dwell on the ground (Sazima, 1992; OAVM, pers. obs.). Although *B. insularis* can also dwell on the ground, individuals of this species spend a significant part of their time in the trees (Marques, 2002b; OAVM pers. obs.). Thus, the smaller body size of female *B. insularis* may be the result of selection for a size more compatible with increased arboreality, as a shorter body

![Fig. 3. Specimens of Bothrops insularis observed in the field in June 2007: female resting on the branch (upper left); one male moved toward this female (upper right); one male inspecting a female (lower left); this male rubbing his “chin” on anterior dorsum of the female (lower right).](image)

![Fig. 4. Litter sizes (based on vitellogenic follicles) of Bothrops insularis (open circles) and Bothrops jararaca (solid circles) in relation to body size of the female.](image)
length and relatively low mass is advantageous for some arboreal snakes (Lillywhite and Henderson, 1993; Wüster et al., 2005).

The seasonal reproductive cycle of the genus Bothrops is usually associated with warm periods of the year. Mating in B. jararaca was recorded only in February and March at the end of the rainy season (Almeida-Santos, 2005). The period of courtship and males searching for females in the field indicate that mating in B. insularis extends from March to August (Amaral, 1921b), including the end of the rainy season and the onset of the dry season (end of autumn–early winter). Field data observations confirm mating in this period and suggest a peak of males searching for females in June and July, in the winter. Thus, mating in B. insularis seems more delayed and longer than that observed for other snakes of the genus Bothrops (cf. Almeida-Santos and Salomão, 2002; Almeida-Santos, 2005). A study to characterize the male reproductive cycle may help to explain the reasons for these differences. One female B. insularis produced unfertilized eggs after eight years maintained alone in captivity, and two years later it gave birth to a normal fully developed offspring and 13 unfertilized eggs (see Almeida-Santos, 2002). Thus, B. insularis may reproduce by facultative parthenogenesis, but long-term sperm storage is also a plausible hypothesis (cf. Booth and Schuett, 2011).

Gestation in B. insularis occurs between October and April when higher temperature (more adequate to embryonic development) is available in QGI. Parturition takes place during periods of rainfall and high availability of food to newborns. Apparently, newborn B. insularis feed upon frogs, lizards, and centipedes, which are abundant between January and April (C. A. Brasileiró, pers. comm., OAVM, pers. obs.). Therefore, the seasonal timing of reproduction of B. insularis is similar to mainland B. jararaca (Almeida-Santos, 2005) as well as other species of the genus Bothrops (Valdujo et al., 2002; Nogueira et al., 2003; Hartmann et al., 2004).

Females of B. jararaca attain larger sizes than do females of B. insularis; thus, the smaller litter size of the latter may be allometrically tied to its smaller body size (Shine, 1994b). Therefore, if we eliminate the body size effect, the number of newborns produced by B. insularis and B. jararaca should be similar. Additionally, females of B. insularis usually have a high number of atretic follicles, which decrease their fecundity even further, although not significantly (see also Hoge et al., 1959).

However, other data show a low fecundity in B. insularis. Data on mainland populations of B. jararaca indicated that approximately half the females reproduce annually (Almeida-Santos, 2005), whereas the frequency of reproductive females of B. insularis is variable and usually lower (0–50%). Moreover, the proportion of newborns in the population of B. insularis (0.6%) is much lower than in mainland B. jararaca in the Atlantic forest. For example, in a snake assemblage studied between 1992 and 1998 in an adjacent mainland locality (Estação Ecológica Juréia-Itatins, 24°18’ to 23°32.5’ and 47°19’ to 47°30’W; Marques, 1998; Marques and Sazima, 2004), the frequency of newborns in B. jararaca sampled was 17.8% (considering also as newborns snakes <350 mm SVL, OAVM, pers. obs). Newborns and juveniles of B. jararaca are prone to occupy arboreal substrates (Marques and Sazima, 2004), which does not make it easy to find them in nature. However, the patterns of substrate use do not seem to differ between newborns/juveniles and adults of B. insularis (OAVM, pers. obs.; unpubl. data). Thus, the difference between both species may not be attributable to the smaller sample of newborns of B. insularis. One alternative hypothesis for the low frequency of newborns observed in B. insularis could be that these dwell predominantly in visually inaccessible microhabitats, such as canopy or underground substrates (the ground on the island is covered in tangled roots with rock). However, the low number of viable vitellogenic follicles as well as the low frequency of reproductive females show that B. insularis has a low rate of recruitment of newborns.

Resource availability is among the most important factors that regulate reproductive frequency output in snakes (Andren and Nilson, 1983; Seigel and Ford, 1987, 1991). Despite being similarly adapted to feed on seasonally abundant migratory birds, the insular Shedaop Pitviper (Gloydius shedaoensis), from northeastern China, and B. insularis are dissimilar in reproductive effort relative to their mainland relatives (Wüster et al., 2005). Although, G. shedaoensis exhibit higher litter mass and larger offspring size than closely related taxa on the Chinese mainland (Sun et al., 2002), B. insularis produces offspring less frequently and of similar size (range 174–285 mm) to those of B. jararaca (190–275 mm, N = 86, see Sazima, 1992). This apparent disparity in reproductive effort was suggested by Wüster et al. (2005) to possibly result from physiological constraints on gravid females or the effects of prolonged inbreeding in B. insularis. However, this pattern could also be explained by

![Fig. 5. Proportion of reproductive females of Bothrops insularis found during the reproductive period (between June and December) at Ilha da Queimada Grande, from 1995 to 2008.](image-url)
differences in prey availability. Shedao Island, situated at 10 km off the coast of China, lies on a major migration route for passerine birds in northern Asia, thus providing high seasonal prey densities for G. shedaoensis (Sun et al., 2002), whereas QGI is situated at 35 km off the coast and has small numbers of migratory passernines (Macarrão, 2010; Marques et al., 2012). Moreover, the density of G. shedaoensis is around four times higher than that of B. insularis (Martins et al., 2008). Thus, prey availability may be lower in QGI than on Shedao Island. In other studies, annual fluctuation in food supply has been shown to influence the proportion of reproductive female snakes (e.g., Madsen et al. 2006). Fluctuating resources may also be responsible for the great year-to-year variation in reproductive frequency of B. insularis if annual variation in the numbers of migrating birds influences resource availability to snakes. A long-term study, quantifying the annual availability of prey and response of snake population parameters, could clarify the effect of food supply on annual reproductive rates of females of B. insularis.

Bothrops insularis is considered as critically threatened, and there is strong evidence that its population size is decreasing (Martins et al., 2008). Suspected causes for population decrease include decline in the quality of the habitat and illegal removal of snakes from the island (Martins et al., 2008). However, conservation management focusing only on the island is not enough because food intake is essential to ensure reproductive events in this snake. Most of the migratory passernines come from the adjacent Atlantic forests on the mainland (Macarrão, 2010; Marques et al., 2012). Thus, taking measures to guarantee the conservation of the Atlantic forest may also be essential for maintaining a viable population of the Golden Lancehead at QGI.

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