

REPRODUCTIVE BIOLOGY OF THE "GLASS SNAKE" *OPHIODES FRAGILIS* (SQUAMATA: ANGUIDAE) IN SOUTH-EAST BRAZIL

L. PIZZATTO

Pós-graduação em Ecologia, Depto. de Zoologia, Universidade Estadual de Campinas, Campinas, SP, Brazil

The reproductive cycles of tropical lizards can be continuous or seasonal, depending on a wide variety of factors, some of which are historical. Most studies on reproductive biology of lizards have been conducted on species in the Iguanidae, Gekkonidae, Teiidae, Lacertidae, and Scincidae. The few studies on anguid lizards have been on various alligator lizards and one species of *Ophisaurus*. This study presents data on sexual maturity, adult body size, neonate body size and body mass, fecundity, and male and female reproductive cycles of a population of *Ophiodes fragilis* from São Paulo and Paraná states in south-east Brazil. Females are larger and attain sexual maturity at a larger SVL than males. The female reproductive cycle is highly seasonal with secondary vitellogenesis starting in the middle of the rainy season (February). Embryos in early stages of development occur in the dry season (June) and parturition occurs at the transition from the dry season to the rainy season (August-December). Clutch size averages 7.5 young and is not related to maternal female size as in many other lizard species. Neonates were 33-57 mm in SVL and 0.45-0.85 g in mass. The residual volume of testes does not vary throughout the year suggesting a continuous spermatogenic cycle. Diameter of the deferent duct is largest from January to March (onset of the rainy season), suggesting that even though males produce sperm continuously, they store sperm until mating season.

Key words: legless lizard, reproduction, clutch size, reproductive cycle, spermatogenic cycle

INTRODUCTION

In temperate areas lizard reproduction is seasonal with mating and egg-laying often occurring from spring to summer (Fitch 1970). Some species mate in autumn and females of some of these species can store sperm over winter (Fox, 1963; Conner & Crews, 1980; Kwait & Gist, 1987). However, tropical lizard species reproduce continuously in some areas (Inger & Greenberg, 1966; Fitch, 1982; James & Shine, 1985; Vitt, 1986; Patterson, 1990) and seasonally in some areas where rainfall is seasonal (Fitch, 1982; James & Shine, 1985; Patchell & Shine, 1986; Clerke & Alford, 1993; Vrcibradic & Rocha, 1998). Male and female reproductive cycles can differ with males producing sperm through the year whereas females may produce eggs only seasonally (Clerke & Alford, 1993; Wilhoft, 1993a,b; Van Sluys *et al.*, 2002). In addition, intraspecific variation in the reproductive patterns may occur across different latitudes or climatic areas (Fitch, 1982; Clerke & Alford, 1993). More recent analyses of existing squamate reproductive data demonstrate that a considerable phylogenetic signal exists in lizard reproductive strategies. Species within clades tend to be more similar to each other in their reproductive strategies than they are to species in distant clades (Dunham and Miles, 1985). The ability to discern evolutionary strategies in lizard reproduction requires reasonable data on reproductive variables of species in all major clades. One

shortcoming of the study by Dunham and Miles (1985) and earlier studies (Tinkle *et al.*, 1970) has been a lack of data on several important lizard clades, such as the Anguidae.

This kind of gap is also seen in Neotropical areas. Although several studies about lizard reproduction are available in this region, they are limited to only some families: Polychrotidae (Vitt & Lacher Jr., 1981), Scincidae (Vitt & Blackburn, 1991; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999), Tropiduridae (Rocha, 1992; Van Sluys, 1993; Vieira *et al.*, 2001; Van Sluys *et al.*, 2002; Wiederhecker *et al.*, 2002) and Gekkonidae (How *et al.*, 1986; Vitt, 1986). The few reproductive studies on anguid lizards have been conducted on various alligator lizards (Vitt, 1973; Goldberg, 1972, 1975) and one species of *Ophisaurus* (Fitch, 1989), all in the northern temperate zone.

The genus *Ophiodes* (Anguidae) is exclusively Neotropical and are distributed in the east Andes, central, east and south-east South America. The genus contains about seven species of limbless, elongate lizards (Martins, 1998). *Ophiodes fragilis* Raddi, 1820 (after revision of Martins, 1998) occurs in south and south-east Brazil, in coastal south Bahia state, Minas Gerais and Mato Grosso do Sul. In Argentina it is registered in north-east of Misiones (Martins, 1998). This species is common in anthropic areas and, similar to North American *Ophisaurus*, are commonly known as glass snakes. As in other anguids, their cryptozoic habits make it difficult to conduct ecological studies on *Ophiodes*.

In the present study, I present data on the reproductive biology of *O. fragilis* in south-east Brazil, including

Correspondence: L. Pizzatto, Depto. de Zoologia, Universidade Estadual de Campinas, CP. 6109, cep. 13083-970, Campinas, SP, Brazil.
E-mail: ligia_oceanica@yahoo.com

size at sexual maturity, body size of males and females, sexual dimorphism, reproductive cycles, fecundity and neonate size.

MATERIAL AND METHODS

A total of 115 (32 females, 42 males and 40 juveniles) specimens were examined from the collections of the Museu de História Natural da Universidade Estadual de Campinas (ZUEC) and Museu de História Natural do Capão da Imbuia (MHNCI). Areas where these specimens were collected are covered mainly by open vegetation, disturbed areas and plantations in São Paulo and Paraná states, south-east Brazil (between latitudes 19.9° and 26.6°S). Climate is seasonal. The warm and rainy season extends from October to March and the cold and dry season from April to September (Nimer, 1989). During the rainy season mean temperature varies from 21 to 24°C and mean rainfall varies from 123 to 240 mm. In the cold and dry season mean temperature varies from 17 to 22°C and mean rainfall varies from 36.8 to 121 mm (Nimer, 1989).

The following data were taken for each specimen: (1) snout-vent length (SVL – to the nearest 1 mm); (2) sex; (3) reproductive status: mature or immature. Females were considered mature when the diameter of their ovarian follicles was greater than 3 mm or if they had embryos; males were considered mature if the testes were large and turgid or if the deferent ducts were opaque and convoluted, indicating the presence of sperm (see Shine, 1977); (4) diameter of the largest ovarian follicles or embryos (plus yolk) in females (to the nearest 0.1 mm); (5) embryonic stage (from one to three according to the quantity of yolk, see below); (6) largest, medium and smallest diameters of testes (testicular volume –TV– was estimated using the ellipsoid volume formula $TV = 4/3ab^2$ where a = largest radius, b = smallest radius – cf. James and Shine, 1985); (7) deferent duct diameter near the cloaca (see Almeida-Santos et al., 2003). Development of embryos was categorized as: Stage 1 – just following ovulation (and probably fecundation) when only yolk was visible; Stage 2 – a small embryo could be observed but yolk was still very abundant, and Stage 3 – a large embryo was present and totally developed, without yolk.

Birth data were obtained from gravid females received at Instituto Butantan (IB), São Paulo, Brazil. Pregnancy was detected by palpation and these females were maintained in captivity (at room temperature 19.3–31.0°C) until birth. Food (cockroaches and lycosid spiders) and water were provided *ad libitum*. After birth, each neonate was measured in SVL (to the nearest 1 mm) and weight (to the nearest 0.1 g). Clutch size was estimated based on the number of embryos in preserved specimens. Because captivity may affect gestation and clutch size, data on number of neonate lizards from captive females were not used in fecundity estimate. Neonate SVL was recorded from individuals received at IB on birth time and individuals born in captive. An index of the degree of sexual size dimorphism (SSD) was

“(mean adult SLV of the larger sex/mean adult SLV of the smaller sex) – 1” (cf. Shine, 1994).

Analysis of volume of testes and diameter of deferent duct was made by combining data from each of four seasons: (1) onset of rainy season (October–December), (2) end of rainy season (January–March), (3) onset of dry season (April–June) and (4) end of dry season (July–September). Because only testes volumes were correlated with SVL by seasons, residuals from the regression of testes volume to SVL were used to indicate spermatogenic activity. Diameter of deferent duct was used to indicate sperm release (cf. Volsøe, 1944; Shine, 1977).

RESULTS

SEXUAL MATURITY AND BODY SIZE

The smallest mature female measured 156 mm SVL and the smallest mature male measured 126 mm SVL. Females (SVL = 192.5±28 mm, range 56–300 mm) are significantly larger than males (SVL = 158.9±22.8 mm, range 126–221 mm) ($t=5.87$, $df=1$, $P<0.0001$). The degree of sexual size dimorphism (SSD) was 0.211.

FEMALE REPRODUCTIVE CYCLE

Secondary vitellogenesis starts in February and ovulation occurs just after secondary vitellogenesis, from March until June. At this time much yolk is visible in ova (stage 1). Embryos in stage 2 are found in June and early August (Fig. 1). By the end of August, embryos in stage 3 begin to appear and continue until December (Fig. 1). In September, one female undergoing parturition was received at IB. Another gravid female gave birth in August (after five months and one week in captivity) and another one in October (after four months and two weeks; Fig. 1). Gestation lasts at least five months (Fig. 1) and all embryos within a given female were in the same developmental stage.

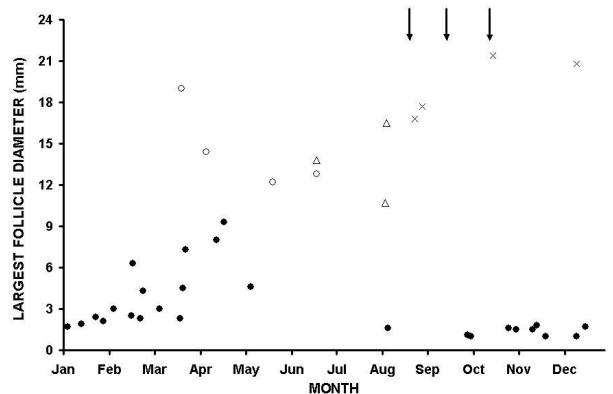


FIG 1. Seasonal variation in the diameter of the largest ovarian follicle in adult females of *Ophiodes fragilis* from south-east Brazil. Solid circles = ovarian follicle; open circles = embryo at stage one of development; triangles = embryo at stage two of development, crosses = embryo at stage three of development; arrows = birth.

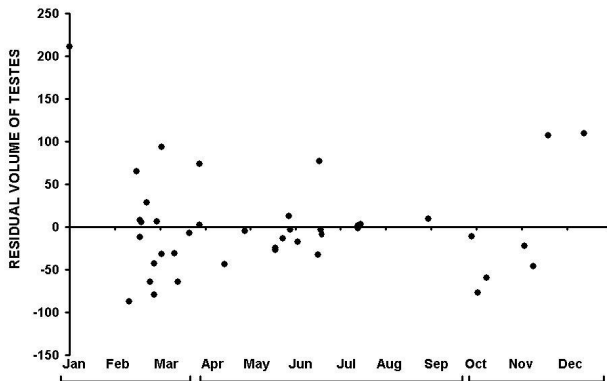


FIG 2. Seasonal variation in the residuals of volume of testes in adult male *Ophiodes fragilis* from south-east Brazil.

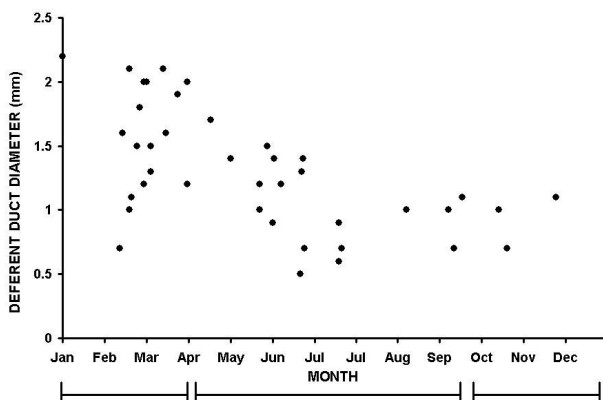


FIG 3. Seasonal variation in the diameter of deferent duct in adult male *Ophiodes fragilis* from south-east Brazil.

FECUNDITY AND NEONATE SIZE

Clutch size averaged 7.5 ± 2.3 embryos (range 5-13, $n=18$) in preserved specimens. In captive specimens (females collected gravid), mean clutch size was 3.6 ± 1.53 (range 2-5, $n=3$). Clutch size was not related to female SVL ($r=0.23$; $P=0.366$, $n=18$). Neonate SVL averaged 44.1 ± 9.4 mm (range 33-57 mm, $n=11$ individuals from three clutches) and weighed 0.69 ± 0.18 g (range 0.45-0.85 g, $n=6$ individuals from two clutches).

MALE CYCLE

Size-adjusted testes volume did not differ among seasons (Kruskal-Wallis test: $H=1.34$, $df=3$, $P=0.718$, $n=41$, Fig. 2). However, diameter of deferent duct decreased significantly from July to December (Kruskal-Wallis test: $H=18.98$, $df=3$, $P=0.0003$, $n=41$) and reached maximum size from January to June (Fig. 3).

DISCUSSION

Females of *Ophiodes fragilis* are larger in SVL than males, as in some other lizard species (and also in snakes) (Patchell & Shine, 1986; Shine, 1994; Vrcibradic & Rocha, 1998). The SSD index to *O. fragilis* was higher than in viviparous skinks of the ge-

nus *Mabuya* (range 0.055-0.145; Rocha & Vrcibradic, 1999) and in many oviparous geckos (range 0.053-0.164; cf. How *et al.*, 1986; Vitt, 1986). However, it is similar to that of some oviparous legless Australian pygopodids (*Lialis burtonis*: 0.242; *Aprasia pulchella*: 0.236; cf. Patchell & Shine, 1986). SSD can be related to both ecological traits and phylogenetic lineage. As in many other lizards, including the oviparous legless anguid *Ophisaurus attenuatus*, clutch size is not significantly related to maternal SVL (How *et al.*, 1986; Patchell & Shine, 1986; Fitch, 1989; Clerke & Alford, 1993; Rocha & Vrcibradic, 1999). In many snakes the primary selective force causing larger SLV in females in relation to males is the potential increase in fecundity (clutch size) associated with increased body size (Shine, 1994). This explanation may apply to lizard species in which clutch size increases with maternal female size, but does not apply to species in which there is no clutch size-female size relationship. In these species, a tradeoff between present and future reproductive success may exist, particularly if females have a high probability of surviving to the next reproductive season (Shine, 1988). As female body size increases, frequency of reproduction may increase thus driving the evolution of female-biased sexual dimorphism in the absence of a clutch size-female size relationship (cf. Shine, 1988).

Clutch size in *O. fragilis* is larger than that in legless oviparous pygopodid lizards but similar to the oviparous legless *Ophisaurus attenuatus* (Patchell & Shine, 1986; Fitch, 1989). Low clutch size in pygopodids probably reflects their origins within the Gekkota, a group of lizards typically having clutch sizes of one or two eggs (Fitch, 1970; Dunham and Miles, 1985; Vitt, 1986). Although relative clutch mass and clutch size may be influenced to some degree by body shape (e.g. Vitt, 1981), clutch size may be conservative in anguid lizards, even when the reproductive mode differs. In captivity, high mortality of *Ophiodes fragilis* embryos (at stage 1) is suggested by the lower clutch size in captive females than in wild ones. Circumstantial evidence suggests that early-stage ova can be reabsorbed. One gravid female (detected by palpation) collected and killed some months after being held captive, contained no vestiges of embryos, and she had regressed ova in the oviducts.

The female reproductive cycle in *Ophiodes fragilis* was highly seasonal, as is expected for viviparous reptiles. Oviparous lizards usually tend to lay eggs from late spring to early summer both in temperate (Mayhew, 1963; Fitch, 1989) and tropical areas (How *et al.*, 1986; Patchell & Shine, 1986; Rocha, 1992; Clerke & Alford, 1993; Van Sluys, 1993; Vitt, 1973). Hatching may occur in late summer or early autumn. However, in *O. fragilis* secondary vitellogenesis and ovulation starts in late summer and early autumn, pregnancy lasts at least five months (March to August) and parturition starts in late winter (August). This pattern is similar to that of some tropical viviparous skinks of the genus *Mabuya* (Vitt & Blackburn, 1991; Vrcibradic & Rocha, 1998; Rocha & Vrcibradic, 1999) and differs significantly from that of

the viviparous anguid *Gerrhonotus coeruleus principis* in temperate areas (Vitt, 1973).

Barbosa *et al.* (1991) recorded parturition of a captive female *O. cf. striatus* collected in Rio de Janeiro State in October. Although this record is consistent with the present study, Barbosa *et al.* (1991) did not describe the specimen in his work. Because *O. striatus* is a large complex of species, in which *O. fragilis* was included (Martins, 1998) it is not possible to recover its actual identification. Marques & Sazima (2003) recorded parturition of two *O. fragilis* (O.A.V. Marques, pers. com) collected in the Atlantic forest in November. Additionally, I recorded parturition, in captivity, of an individual from Minas Gerais State. This female was collected on 22 July and gave birth to six lizards on 4 October indicating that its reproductive period is similar to that of other populations close to the studied area.

Although some tropical species have seasonal spermatogenesis (Rocha, 1992; Vrcibradic & Rocha, 1998) many others produce sperm continuously (Van Sluys, 1993; Van Sluys *et al.*, 2002), similar to *O. fragilis*. However, in this species, mating appears to be seasonal, occurring at the end of the rainy season (at least from February to March) as indicated by the increase in deferent duct diameter. Mating during this season is synchronous with fecundation and thus, sperm storage by females is not essential to reproductive cycle in this species.

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APPENDIX 1

SPECIMENS EXAMINED

MHNCI: 198, 808, 849, 1438, 1697, 1699, 2075, 2076, 2106, 5166, 6756, 6984, 6985, 7119, 7227, 7272, 7394, 7442, 7660, 7761, 8156, 8228, 9026, 9164, -641.
 ZEUC: 2511 – 2548, 2550 – 2569, 2572, 2574, 2576, 2610 – 2614, 2616 – 2618, 2807 – 2829.