INTRODUCTION

Continuous reproductive cycles in snakes are expected in tropical regions without a well-marked dry season (Fitch, 1982; Saint-Girons, 1982; Vitt, 1983; Seigel & Ford, 1987), although several tropical species show seasonal reproduction and intra-sex synchrony (Fitch, 1982; Fowler et al., 1998; Marques, 1996, 1998, 2002; Marques & Puorto, 1998). This synchrony may be a strategy by which individuals maximize their reproductive success (see Ims, 1990). Mating periods are unknown for most snake species and male reproductive cycles are less understood than those of females (Saint-Girons, 1982). Moreover, there is very little information on either the sperm storage organs of the male or the seasonal location of the sperm (Scheutt, 1992). Although mating and fertilization in snakes may occur in the same period (e.g., Marques, 1996), in several species mating time does not coincide with fertilization (Fox 1956; Darevsky, 1971; Halpert et al., 1982; Scheutt, 1992; Almeida-Santos & Salomão, 1997; Almeida-Santos et al., 2004). In addition, when spermatogenesis is not coincident with vitellogenesis (see Jordão, 1996; Bizerra, 1998) it is very difficult to determine mating periods.

The Atlantic coral snake, Micrurus corallinus, is a common snake in the Atlantic forest domain in south-eastern Brazil (Marques et al., 2004). It occurs in dense ombrophilous and semi-deciduous seasonal forests in Brazil, Paraguay, Uruguay and Argentina (Campbell & Lamar, 2004). The female reproductive cycle is seasonal, with vitellogenesis and mating occurring in the early rainy season, oviposition in mid–rainy season and hatching at the end of the rainy season and in the early dry season (Marques, 1996). However, nothing is known on the reproductive cycles of males. Here we present data about testicular activity, sperm storage in the deferent ducts, reproductive aggregation, and relation of activity to reproductive cycles of males and females.

MATERIAL AND METHODS

A total of 187 M. corallinus males were examined from the collections of the Instituto Butantan and Museu de História Natural da Universidade Estadual de Campinas. The sample included only adults (larger than 440 mm in snout-vent length – see Marques 1996) from São Paulo State, south-eastern Brazil (between 19.7°N, 25.3°S, 53.2°W and 44.2°E). The following data were taken from each specimen: (1) snout-vent length (SVL); and (2) testis length and diameter of a deferent duct at its distal end (see Fig. 1), both recorded on the right side. Spermatogenic cycles were determined relating the testicular length to spermatogenic activity (Volsøe, 1944; Shine, 1977; Seigel & Ford, 1987). Similar correlation has been shown between morphology of the deferent duct and sperm storage (Yokoyama & Yoshida, 1993; Sever et al., 2002; Almeida-Santos, 2005).

As testis length was related with SVL ($R^2 = 0.19$, $P < 0.0001$), we used the residuals from the linear regression of testis length and SVL as measures of relative testis length (see Shine, 1992). Deferent duct diameter was not related to SVL ($R^2 = 0.03$, $P = 0.300$), then, the residuals were not used in this case. Variation in relative length of testis and deferent duct diameter was analyzed.
by ANOVA and a post hoc Tukey test (Sokal and Rohlf 1995), to infer spermatogenic cycle (see Volsøe, 1944; Shine, 1977; Seigel & Ford, 1987) and sperm storage (Yokoyama & Yoshida, 1993; Sever et al., 2002; Almeida-Santos et al., 2004), respectively.

Fifteen specimens from the sample used for morphological measurements (four from spring, three from autumn, five from winter and three from summer) were selected randomly and the right testis and distal region deferent duct were removed, dehydrated in ethanol, and embedded in paraffin. Histological sections were cut at 5 µm and stained in hematoxylin/eosin. Sections of the testes and deferent duct were examined to determine the stage of the testicular cycle and the presence or absence of spermatozoa, respectively.

Data on the female reproductive cycle and snake activity were obtained from Marques (1996), where seasonal activity was inferred from collection data (see Marques et al., 2001 for discussion about this method). In the present study these data on seasonal activity are combined in different seasons which represent the major climatic variations in the study area (Nimer, 1989). In south-eastern Brazil the rainy season comprises austral spring (October – December) and summer (January – March), whereas the dry season comprises autumn (April – June) and winter (July – September). The number of males and females per season were compared by Chi-squared test to infer variation in activity ($H_0$ = there is no variation in number of snakes per season; Zar 1999). Records on mating aggregation in nature were also used in the present study.

### RESULTS

The adult males examined averaged 523.5 mm SVL (SD=78.9 mm, range 462-743 mm, n=187). Testes reach their maximum relative length during autumn and their minimum in spring ($F=3.206$, $P=0.025$, df=3; Fig. 2), whereas distal deferent ducts are at their smallest diameters in summer–autumn and increase in winter–spring ($F=14.61$, $P<0.0001$, df=3; Figs. 1,3). In this study, we distinguished two main stages of testicular cycle in *M. corallinus*, regression and spermiogenesis. In the spring, the epithelium was exhausted and highly disorganized, with little spermatogonia and sperm in the lumen, characterizing the regression phase. During the summer, the epithelia were starting to be reorganized, there were more spermatogonia than in the previous season, but no sperm were found in the lumen. During the autumn and winter, we recorded the spermiogenesis phase, when seminiferous tubes were highly organized and the lumina were lined by rows of metamorphosing spermatids and spermatozoa. Mature spermatozoa were the predominant cells in the seminiferous tubules. The deferent duct was straight and no sperm were observed in its lumen during the autumn. During the winter, the deferent duct was slightly convoluted and little sperm was found,
whereas in the spring, the deferent duct was convoluted and completely full of sperm, and then, in the summer, it was slightly convoluted again and the amount of sperm started to decrease.

The activity in both males and females peaks in spring (males: $\chi^2=73.1$, df=3, $P<0.0001$; females: $\chi^2=73.6$, df=3, $P<0.0001$). The number of females collected outnumbered males throughout year, but differed significantly only in summer ($\chi^2=3.7$, df = 1, $P=0.055$).

A mating aggregation of *M. corallinus* was observed in the field on 28 October 1999. The group consisted of a male with an everted hemipenis and two females (P. B. de Souza, pers. comm.). Another group composed of two males and one female was observed on 17 December 1999 (G. Ferranti, pers. comm.). In both cases females had vitellogenic follicles, and deferent ducts of males had large diameters.

**DISCUSSION**

In post-nuptial or aestival spermatogenesis (Type I, according to Schuett, 1992) many snakes exhibit maximum testes sizes in autumn – reflecting maximal spermatogenic activity – and mating occurs early in the following spring, utilizing sperm stored over winter in the deferent ducts (Saint-Girons, 1982; Seigel & Ford, 1987). Some authors suggest that sperm storage in the male reproductive tract indicates prolonged mating time (Quinn, 1979; Jackson & Franz, 1981; Johnson et al., 1982; Bull et al., 1997) or an adaptation to the timing of the mating season (Shine, 1977; Garstka et al., 1982; Saint-Girons, 1982; Mitchell & Zug, 1984). The latter seems to be the case in *M. corallinus*: sperm produced largely in autumn is stored in the distal end of deferent ducts (as in the viperid *Protobothrops flavoviridis* – see Yokoyama & Yoshida, 1993) until the mating season (spring), when ovarian follicles in females are in secondary vitellogenesis (see Marques, 1996), and testicular size is minimal. Therefore, diameter of the distal deferent duct is a good indication of the mating season, especially when reproductive cycles are post-nuptial. The reproductive cycle of *M. corallinus* males is post-nuptial (or Type I, see Schuett, 1992), whereas the female cycle is pre-nuptial because vitellogenesis coincides with mating (Garstka et al., 1982; Saint-Girons, 1982; Seigel & Ford, 1987). Although gametogenesis in both males and females is not simultaneous, the co-ordination of their reproductive cycles is guaranteed by sperm storage in males.

Our results are similar to those recorded for *M. tener* from Texas, in which vitellogenesis and oviposition occur during spring (Quinn, 1979) and testicular recrudescence peaked in autumn. Quinn (1979) suggests that sperm is stored by females in the oviducts, although the mating time is still uncertain. In Florida, *M. fulvius* has secondary vitellogenesis in late winter–spring and egg-laying in late spring–summer, whereas testes size is maximum during autumn and decrease in spring (Jackson & Franz, 1981). Thus, the same pattern of intra-sex synchrony and inter-sex co-ordination is
seen in other species and populations of *Micrurus* (pers. obs.). Species of *Micrurus* seem to have both vitellogenesis and spermatogenesis adjusted to the same season of the year in different areas of the same latitude (see Werler, 1951; Campbell, 1973; Quinn, 1979; Jackson & Franz, 1981; this study). However, in equatorial areas the reproductive pattern can differ. *Micrurus nigrocinctus* in Costa Rica presents a more extensive female cycle, from five to seven months of vitellogenesis and oviposition (see Solórzano & Cerdas, 1988; Goldberg, 2004). Moreover, Goldberg (2004) also recorded sperm production throughout the year (despite the lack of data in some months), for this species. Detailed investigation on spermatogenesis and vitellogenesis in other species of *Micrurus* are essential to characterize the reproductive patterns and understand the climatic influence on the reproduction of *Micrurus*.

Field observations of *M. corallinus* mating were recorded in October and November (Marques, 1996), and during the mating season, ritual combat was not observed. Ritual combat among males occurs in several snake species during the mating season (Gillingham, 1987; Greene, 1997) and was recently described for some tropical species (Almeida-Santos et al., 1999; Almeida-Santos & Marques, 2002), including *Micrurus* (Almeida-Santos et al., 1998). This behaviour seems to be common in species in which males are larger than females (Shine, 1978, 1994). However, in some species, both from tropical or temperate areas, more than one male may court a female without combat (see Slip & Shine, 1988; Greene, 1997; Feio et al., 1999; Rivas, 1999), although some agonistic interaction generally occurs (Capula & Luiselli, 1997). Our findings indicate that *M. corallinus* aggregate for mating and it is possible that there is no combat among males, as females are larger than males (Marques, 1996). In the genus *Micrurus*, combat ritual is recorded only for *M. frontalis* (Almeida-Santos et al., 1998), in which sexual dimorphism is apparently absent (Roze, 1996; O. A. V. Marques, pers. obs.). Mating aggregations in tropical snakes probably are more common than generally thought and observations on aggregations such as that reported for *Imantodes cenchoa* (Doan & Arriaga, 1999; Rivas, 1999), although some agonistic interaction generally occurs (Capula & Luiselli, 1997). Our findings indicate that *M. corallinus* aggregate for mating and it is possible that there is no combat among males, as females are larger than males (Marques, 1996). In the genus *Micrurus*, combat ritual is recorded only for *M. frontalis* (Almeida-Santos et al., 1998), in which sexual dimorphism is apparently absent (Roze, 1996; O. A. V. Marques, pers. obs.). Mating aggregations in tropical snakes probably are more common than generally thought and observations on aggregations such as that reported for *Imantodes cenchoa* (Doan & Arriaga, 1999) may actually be mating aggregations.

Seasonal activity of *M. corallinus* seems to be strongly influenced by the reproductive cycle, although other factors may also have an impact on activity patterns (see Marques, 1996; Marques et al., 2001). The increase of activity in females during spring is probably due to vitellogenesis and mating when thermoregulation time is longer (see Marques, 1996; Shine, 1979). The high number of females in summer occurs just after oviposition when they may forage for food to replace energy lost in egg reproduction. In males, spermatogenesis is uncoupled from mating and testicular recrudescence occurs in summer, after sexual activity ceases. Thus, male activity decreases in summer and autumn (Fig. 3) when energy is needed for sperm production, which may be costly (Olsson et al., 1997). Sperm are released from the testes during autumn and stored in deferent ducts over winter until spring when they mate. Thus, male activity increases in spring probably due to their searching for females (Duvall et al., 1992), which could originate aggregation and perhaps competition during mating. Aggregation and female reproductive synchrony could favour polygyny rather than monogamy in *M. corallinus*, probably the most common snake mating system (Duvall et al., 1992).

ACKNOWLEDGEMENTS

We thank Ivan Sazima, Richard Shine, and three anonymous referees for critical review of the manuscript. João C. Ferreira and Valdir J. Germano provided assistance in the laboratory. The CNPq provided fellowships to OAVM (300073/99-2). This study is part of the project “História Natural, Ecologia e Evolução de Vertebrados Brasileiros” founded by the FAPESP (grant 00/12339-2).

REFERENCES


Accepted: 7.3.06