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THE TESTICULAR CYCLE OF CAPTIVE *TUPINAMBIS MERIANAE* LIZARDS IN A TEMPERATE ENVIRONMENT

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R E S U M E N. — Las poblaciones de *Tupinambis merianae*, que habitan las regiones templadas y subtropicales, muestran un comportamiento marcadamente estacional con períodos equivalentes de actividad e hibernación. Correspondientemente, la reproducción en estos lagartos es un fenómeno cíclico caracterizado por una corta actividad sexual primaveral. Este trabajo examina los cambios testiculares que ocurren durante el ciclo reproductivo de especímenes de *Tupinambis merianae* criados en un ambiente templado. Evalúa la participación de la porción renal sexual como glándula sexual secundaria. En otoño e invierno, el testículo exhibe una gametogénesis discontinua. La espermatocitogénesis ocurre en otoño dando lugar a una espermiogénesis precoz abortiva, que concluye en los meses fríos. En la primavera temprana, la gónada reinicia su actividad espermiogénica alcanzando prontamente un pico de máximo crecimiento y abundante producción de esperma. El clímax testicular ocurre brevemente después de la hibernación y coincide con un período de cópulas de alrededor de un mes (octubre). En ese período, el epidídimo considerablemente dilatado se encuentra revestido por un epitelio cilíndrico y contiene grandes masas de esperma. Simultáneamente, la porción sexual de los túbulos renales exhibe células columnares hipertróficas cargadas de grandes gránulos citoplasmáticos PAS (+). Pronto la actividad reproductiva cesa y da lugar a una fase de involución gonadal, que se extiende a través del resto de la primavera y verano (noviembre-febrero), indicando la existencia de un único evento reproductivo al año. Las observaciones se discuten en relación con los factores climáticos y las características biológicas del grupo.

Palabras clave: *Tupinambis*, ciclo testicular, espermatogénesis, reproducción.

A B S T R A C T. — *Tupinambis merianae* populations living in temperate and subtropical regions show a distinctly seasonal behaviour, with equivalent periods of activity and hibernation. Correspondingly, reproduction in these lizards is a cyclic phenomenon, characterized by a short spring sexual activity. This work examines the testicular changes that occur during the reproductive cycle of *Tupinambis merianae* specimens raised in a temperate environment. The involvement of the kidney sexual portion as a secondary sexual gland is also considered. In autumn and winter, the testicle exhibits a discontinuous gametogenesis. Spermacytogenesis takes place in autumn, giving rise to a precocious abortive spermiogenesis which concludes at cool months. At early spring, the gonad restarts its spermiogenetic activity attaining promptly a peak of maximal growth and abundant sperm production. The testicular climax occurs shortly after hibernation and coincides with a mating period of about a month (October). At that period, the quite enlarged epididymis is lined with a cylindrical epithelium and contains large sperm masses. Simultaneously, the sexual portions of kidney tubules display hypertrophic columnar cells filled with large PAS (+) cytoplasmic granules. Soon, the reproductive activity ceases and makes way for a phase of gonadal involution which extends through the rest of spring and summer (November-February), indicating the existence of a single reproductive event per year. The observations are discussed in connection with climate factors and biological features of the group.

Key words: *Tupinambis* lizards, testicular cycle, spermatogenesis, reproduction.

INTRODUCTION

Genus *Tupinambis* (Squamata: Teiidae), from which at least five species were recognized (Ávila-Pires, 1995; Colli *et al.*, 1998; Fitzgerald *et al.*, 1999), comprises a group of large terrestrial lizards of the South America plain (Presch, 1973), living in a wide range of climates, from tropical to temperate ones.

Tupinambis merianae and *Tupinambis rufescens* represent the southernmost populations reaching the north of Patagonia (Cei and Scolaro, 1982). With diurnal habits, robust constitution and total lengths of about 1.2 m in their adult state, they have been described as active hunters, performing an important role in the trophic chain (FAO-PNUMA, 1985).

In subtropical and temperate environments, they show a strong seasonal behavior with equivalent periods of activity and hibernation (Donadío and Gallardo, 1984; Mercolli and Yanosky, 1990; Noriega *et al.*, 1996). From a reproductive point of view, *T. merianae,* as *T. rufescens,* displays an oviparous reproduction with a single annual laying of 30 to 40 eggs (Donadío and Gallardo, 1984; Noriega *et al.,* 1996).

Historically, both species constituted a valuable resource for the indigenous communities, which took advantage of their leather, meat and fat (Donadío and Gallardo, 1984; Norman, 1987). But since the 1970s, they have been intensively exploited for the leather traffic, being included in the Appendix II of CITES. For Argentina and Paraguay, the current extraction quota has been estimated in 1,3 millions skins/year (Fitzgerald *et al.*, 1999).

Fortunately, successful captive breeding projects indicate the possibility of a sustainable use of these animals, as well as the restoration of their natural populations (Mercolli and Yanosky, 1990; Noriega *et al.*, 1996). Nevertheless, a good handling of the group still requires a basic understanding of its reproductive biology.

The present paper examines the testicular cycle and kidney sexual segment of *Tupinambis merianae* in a captive breeding program, carried out in a temperate environment.

MATERIAL AND METHODS

The studies were performed with *Tupinambis merianae* specimens from «El Gringo» hatchery, located in Sa Pereira, province of Santa Fe, Argentina. They were raised in outdoors enclosures, fenced by masonry walls, 1.2 m high, containing refuges and shelters. The feeding, *ad libitum*, consisted mostly of bovine meat supplemented with station fruits, cereals and eggs. The reproductive stock occupied a surface area of 8 m^2 per individual in a 1:5 male to female relationship.

Male individuals were collected at month intervals (2-4 individuals per month) during the period of June 1991-February 1993. A further collection was performed in December 1995. This study comprises 38 fertile specimens, with a snout-vent length of more than 35 cm, length at which they are sexually mature (unpublished observation).

After being killed, the animals were measured and weighed. Inmediately afterwards, they were dissected and the testes, epididymes and kidneys were fixed in a Duboscq-Brasil solution for 24-48 hs. After fixation, the samples were preserved in 80% alcohol.

The reproductive state was estimated weighing the gonad to the nearest 0.01 g. Testicle volume was also calculated, measuring the main and minor diameters with a caliper to the nearest 0.05 mm and applying the ellipsoid formula: $V = 4/3\ p\ a^2\ b$; where $a = ½$ minor diameter and $b = ½$ main diameter (Beyer, 1982). Any gonadal size alteration which may have been caused by fixation was dismissed on the assumption that it was similar for all the samples.

For the histological studies, samples

were prepared with conventional paraffin embedding techniques. They were serially sectioned at 7µm and stained with haematoxilin-eosin (H-E); Heidenhain's azocarmin anilin (Azan) and periodic acid Schiff (PAS).

To quantify the relationship between gonadal and body size, the Pearson correlation coefficient was used. To establish statistical differences of the means, the analysis of variance (ANOVA) and Tuckey HSD Multiple Comparison Test were performed. To homogenize variances, the logarithm transformation of gonad and body dimensions was used.

RESULTS

The locality of Sa Pereira in the Argentinean plain, where the samples were taken, exhibits a temperate climate with an annual mean temperature of 19°C. Precipitations amount to 1100 mm per year and occur mainly in spring and summer. Figure 1 shows the seasonal variations of temperature and rainfalls in Rafaela, 30 km N away from Sa Pereira.

TESTIS

Morphometric changes. — The high correlation between variations of testicular mass and volume, r =0.91 (*log* testicular mass = -1.25 + 0.55 *log* testicular volume), allowed the use of either value. For this presentation, we used the testicular mass because of its direct measurement.

On the other hand, the low correlation coefficients between the gonad (mass or volume) and body (mass or snout-vent length) dimensions did not sustain the use of a gonadosomatic index. Therefore, the testicular cycle of *Tupinambis merianae* was expressed as a logarithmic function of the absolute gonadal mass (Fig. 2).

After its maximal involution at the end of summer (February), the gonad exhibits a slight mass increase during autumn (March-May). At early spring (September), the mass abruptly rises to its greatest value in October, when mating can be seen on the farm. Subsequently, the gonad loses mass progressively up to February, with a maximal testicular atrophy (Fig. 2).

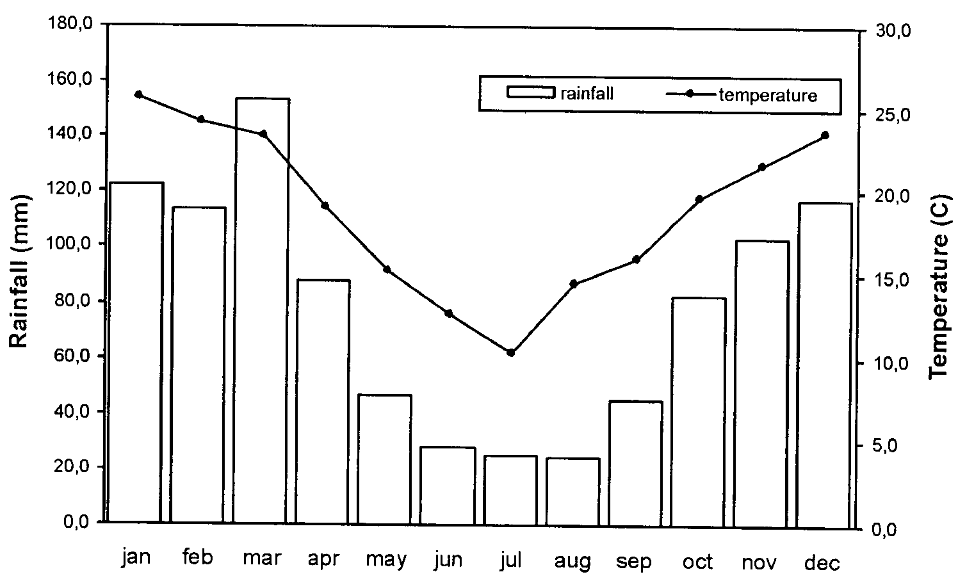

Figure 1. Monthly means of temperatures and precipitations in the locality of Rafaela. Data collected between 1990 and 1993.

The ANOVA indicates the existence of statistical differences among the monthly means of testicular mass log, being $F_{10, 53} = 20.24$, and $p < 10^{-6}$. The Tukey Multiple Comparison Test between pairs of means shows that the mass in October differs significantly from the remaining months (p<0.01), excepting September. In addition, the test shows that mass in February is statistically different from the other months, excepting March and December (p<0.05).

Histological changes. — In autumn (March), the gonad starts its recovery (recrudescence) by forming spermatocytes I and II, which markedly enlarges the thickness of the seminiferous epithelium (Fig. 3 A, compare with Fig. 3 H).

Around the end of autumn, the seminiferous tubulae appear loaded with sperm cells (Fig. 3 B). The epidydimis, little developed and lined with a columnar epithelium, also contains sperm aggregates (Fig. 4 A). However, in early winter (June-July) the sperm cells in the seminiferous tubulae are replaced by celular residues, which would indicate a detention of the spermiogenic process. Nevertheless, the germinal epithelium consists of several cell layers, possibly differentiating spermatogonia (Fig. 3 C). Towards the end of winter (August) the seminiferous epithelium is composed only by several layers of spematocytes I and II (Fig. 3 D).

At the beginning of spring (September), well developed seminiferous tubulae enter in a new spermiogenetic process, showing spermatocytes I y II, spermatids and scarce sperm groups (Fig. 3 E). These changes, together with an increase of the gonadal size, anticipate the end of hibernation and the onset of the reproductive phase.

In October, in coincidence with a relatively brief and intense period of sexual activity, the gonad reaches its maximal development, showing a predominance of spermatides and sperms (Fig. 3 F; G).

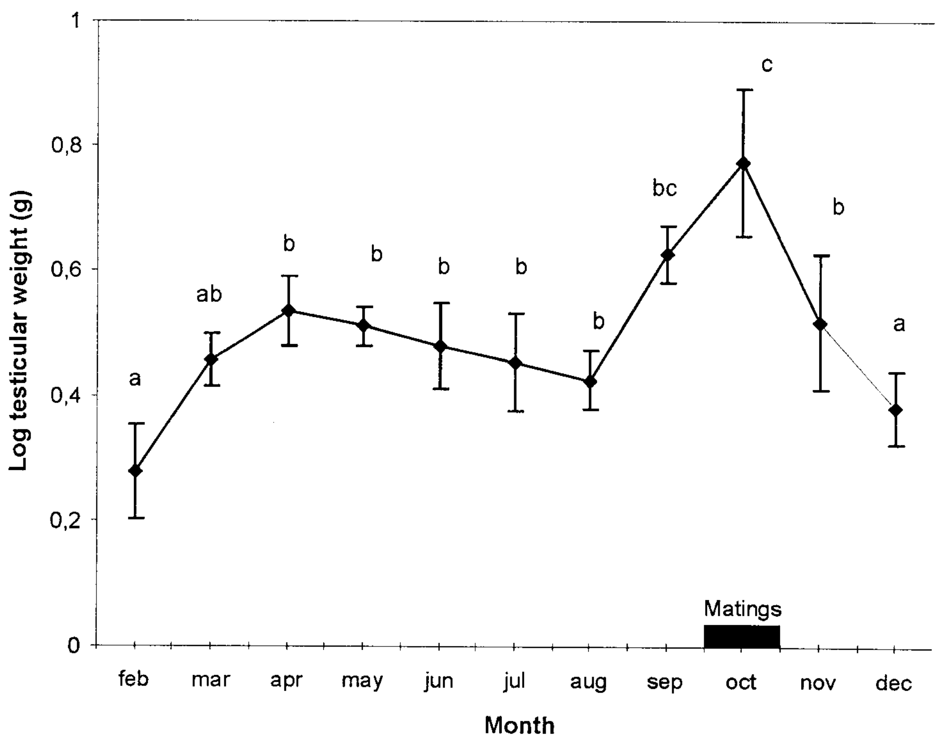

Figure 2: Annual cyclic variation of the testis mass in *Tupinambis merianae*.

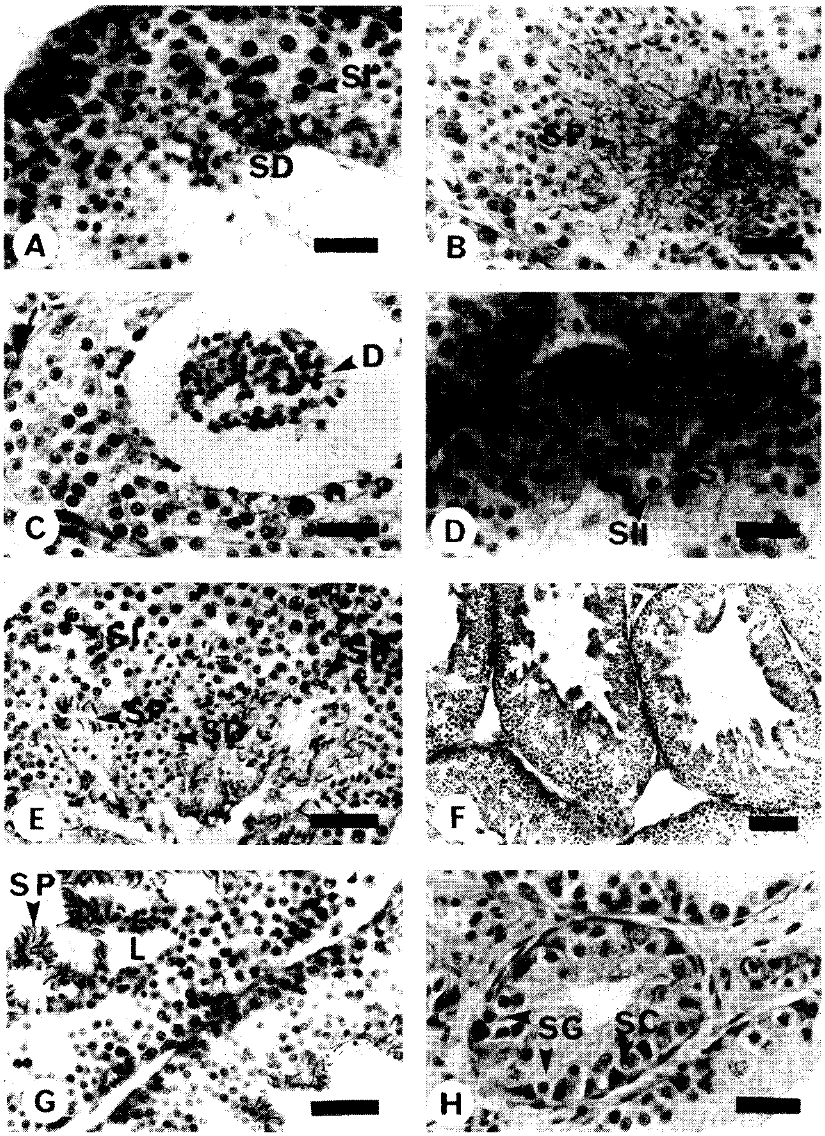

Figure 3: Histological changes in testis.
A. Seminiferous tubule in recrudescense at the beginning of autumn (March), predominantly composed by spermatocytes I and II, and some spermatids.
B. Seminiferous tubule showing a precocious spermiogenesis at the end of autumn.
C. Cell debris in a seminiferous tubule at the beginning of winter (July).
D. Germinal epithelium at the end of winter (August), formed by spermatocytes I and II.
E. Seminiferous tubule at the onset of functional spermiogenesis in spring showing a complete spermatogenic series (September).
F. General aspect of seminiferous tubules at the maximal gonadal development (October).
G. Detail of the wall of seminiferous tubules showing notorious intercellular spaces and clustered sperms lining the tubular lumen (October).
H. Atrophied testicular cord during the greatest gonadal regression (February). Sertoli and gonial cells represent the main components.
Abbreviations: SG spermatogonia; SI primary spermatocyte; SII secondary spermatocyte; SD spermatid; SP sperm; SC Sertoli cell; D cell debris; L lacuna
Scale bars: **A**; **B**; **C**; **D**; **E** **G**; **H** =. 30 mm; **F** = 100 mm.

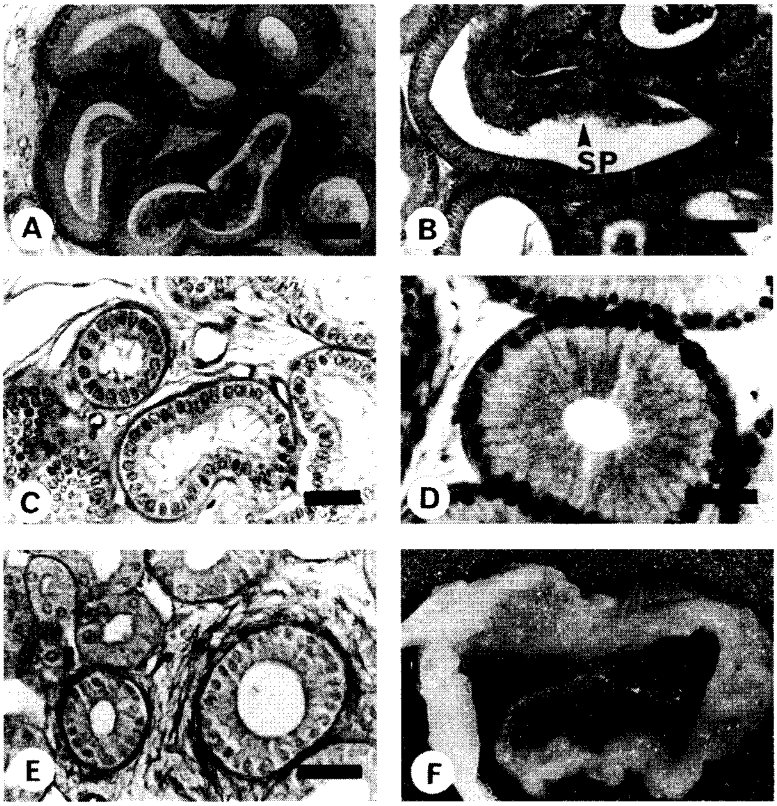

Figure 4: Histological changes in epididymis and kidney sexual segment.
A. Epidydimis at the autumnal spermiogenesis containing sperm masses.
B. Enlarged epidydimis during the sexual interactions (October). Note the ample lumen and the large sperm masses.
C. Epidydimis at the gonadal regression stage, showing a lining of cubic cells with central nuclei (February).
D. Hypertrophic kidney sexual segment during the sexual phase. Notice the columnar cells showing basal nuclei and abundant PAS (+) cytoplasm granules.
E. Involuted kidney sexual segment (February).
F. Mucous material released during the sexual interactions.
Scale bars: **A**; **B**; **C**; **D**; **E** = 30 mm; **F** = 7 mm

The latter are arranged in a continuous layer towards the tubular lumen. This stage is also characterized by the presence of numerous and large intercelular spaces throughout the seminiferous epithelium.

At the same time, the enlarged epidydimis, lined with columnar cells, exhibits large sperm masses (Fig. 4 B).

At the end of the reproductive period (November), the testis starts a regression characterized by a size reduction and the involution of the seminiferous epithelium. Towards the end of summer (February), the atrophied seminiferous tubules seem to be compossed only by spermatogonia and Sertoli cells (Fig. 3 H). The reduced epidydimis shows a cubic epithelium with cytoplasmic projections (Fig. 4 C).

KIDNEY SEXUAL SEGMENT

The male reproductive activity of *Tupinambis merianae* also includes changes in the kidney sexual segment suggesting, as in other lizards (Fox, 1958; Saint Girons, 1984), its role as an accesory sexual gland. During the mating period, this component can be neatly distinguished with a stereomicroscope as a series of white tubular segments, that bestow the organ a stripped aspect. Hypertrophied cells in these conducts display basal nuclei and abundant PAS (+) cytoplasmic granules (Fig. 4 D). Outside the period of sexual activity, these cells lack secretory granules and exhibit a reduced size (Fig. 4 E).

These secretory features are temporally related with the finding of a mucous material on the ground (Noriega *et al.*, 1996) (Fig 4. F).

DISCUSSION

In coincidence with a noticeable seasonal activity, the reproductive cycle of *T. merianae*, as that of *T. rufescens*, corresponds to that of oviparous lizards from temperate climates: with a vernal sexual activity, a single egg laying and a relatively large clutch size (Mercolli and Yanosky, 1990; Noriega *et al.*, 1996).

When testicular changes of *T. merianae* are compared with the available spring and summer samplings of *T. rufescens* (Paz *et al.*, 1993), a similar pattern is seen in both species. The spermatogenesis of *Tupinambis merianae*, with an autumnal spermacytogenesis and a spring functional spermiogenesis, belongs to the «mixed» or «discontinuous» type mentioned for other reptiles (Saint Girons, 1984).

The gametogenesis, occuring during the little propitious seasons of autumn and winter, could obey to physiological constrains imposed by the appreciable body mass of this lizard. The most evident concerns the sustained lethargy, which at these latitudes circumscribes the main reproductive functions: matings, egg laying and incubation to only half a year (Mercolli and Yanosky, 1990; Noriega *et al.*, 1996). Of similar importance is the extended period of incubation (about 70 days) that places births well ahead in summer (middle of January) (Mercolli and Yanosky, 1990; Noriega *et al.*, 1996; Birchard and Marcellini, 1996).

According to the maximal testicular development and mating records (Noriega *et al.*, 1996), the *Tupinambis merianae* sexual interactions in captivity occupies approximately a month (October), a time shorter than that reported for the species in other confinements (Donadío and Gallardo, 1984; Mercolli and Yanosky, 1990) and for *T. rufescens* in the wild (Paz *et al.*, 1993). This difference, however, could be due to the particular conditions of this study, in which a uniform nutritional level and stimulae would have favoured a synchronization of the reproductive behaviour among individuals.

Remarkably, tropical populations of *Tupinambis teguixin*, which are active all year, also reveal a seasonal reproductive pattern (Herrera and Robinson, 2000). The small clutch size, as compared to that observed in subtropical and temperate habitats, induced the authors to consider the possibility of multiple egg laying. In this sense, the authors' graphs seems to suggest a bimodal gonadal function.

In temperate climates, the temperature seems to be the most influential environmental agent on the lizard testicular cycle. While cool temperatures tend to stimulate spermatocytogenesis, the warm ones promote spermiogenesis (Saint Girons, 1984). Similarly, exceedingly cold temperatures could be involved in the hibernal arrest of the early spermiogenetic proccess in *Tupinambis merianae*, phenomenon which probably reflects a filogenetic story in warmer climates.

A link between precipitations and the beginning of the sexual activity was also indicated for *Tupinambis rufescens* (Fitzgerald *et al.*, 1993). However, the

Tupinambis merianae sexual activity, installed shortly after the lethargy, frequently precedes the rainfall season (unpublished observations). Furthermore, the gametogenic cycle is clearly independent of this factor, occuring mainly during hibernation in underground refuges, protected from the few autumn and winter precipitations.

For practical purposes, it would be interesting to see whether the use of controlled thermal conditions in captive breeding programs could modify the seasonal reproductive pattern of *Tupinambis merianae.*

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