Biological Rhythm Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/nbrr20

Seasonal effects of pinealectomy on the locomotor activity rhythm in the lizard Sceloporus torquatus

Manuel Miranda-Anaya [a] , Jesús Corona-Lagunas [a] & Paul A. Bartell [b]

[a] Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, México, DF, 04510

[b] Department of Biology, Texas A&M University, College Station, TX, 77843, USA

Version of record first published: 01 Mar 2007.

To cite this article: Manuel Miranda-Anaya , Jesús Corona-Lagunas & Paul A. Bartell (2007): Seasonal effects of pinealectomy on the locomotor activity rhythm in the lizard Sceloporus torquatus , Biological Rhythm Research, 38:2, 87-93

To link to this article: http://dx.doi.org/10.1080/09291010600902611

Biological Rhythm Research
April 2007; 38(2): 87–93

Taylor & Francis
Taylor & Francis Group

Seasonal effects of pinealectomy on the locomotor activity rhythm in the lizard *Sceloporus torquatus*

MANUEL MIRANDA-ANAYA[1], JESÚS CORONA-LAGUNAS[1], &
PAUL A. BARTELL[2]

[1]*Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, México DF 04510 and* [2]*Department of Biology, Texas A&M University, College Station, TX 77843, USA*

Abstract
Adult lizards (*Sceloporus torquatus*) were used to test whether seasonal differences in the effects of pinealectomy upon the locomotor activity rhythm exist. Animals were field collected and exposed to artificial light–dark cycles and constant temperature in winter and summer. Free running circadian rhythms under constant temperature and dim red light were monitored using infrared light-crossings. The effects of pinealectomy were assessed by analysing the circadian parameters of free running period and activity–rest ratio in constant darkness or light–dark cycles. Results obtained indicate that pinealectomy changes the free running period of locomotor activity rhythm, irrespective of season, while seasonal differences in activity–rest ratio were detected. Our findings support the hypothesis that seasonal regulation of circadian rhythms in lizards is accomplished, in part, via the output of the pineal gland.

Keywords: *Locomotor activity, circadian, lizard, pineal gland*

Introduction

Circadian rhythms allow organisms to fit physiologically and behaviourally to their cyclic environment. Constant feedback among the elements that conform the circadian system to the environment is needed to finetune the timing of the biological clock. Vertebrate circadian organization involves the interaction of multiple circadian oscillators; by understanding the interactions within multi-oscillator systems and with environmental cycles, we may understand the adaptive significance of biological clocks. Iguanid reptiles have a diverse set of neural structures that function as circadian oscillators such as the pineal gland, retina, parietal eye and a homologoue of the suprachiasmatic nucleus of the hypothalamus. Studies have shown that removing the pineal gland in reptiles elicits a wide variety of species dependent effects (reviewed in Tosini et al. 2001; Underwood 2001), indicating that the pineal gland plays a major role in reptilian circadian organization. In the lizards *Sceloporus occidentalis* and *Sceloporus olivaceus*, pinealectomy produces changes in the period of free running rhythms of locomotor activity, may induce splitting in the daily pattern of locomotor activity, and alters

---

Correspondence: Manuel Miranda-Anaya, Departamento de Biologia Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, México DF 04510. E-mail: mma@hp.fciencias.unam.mx

ISSN 0929-1016 print/ISSN 1744-4179 online © 2007 Taylor & Francis
DOI: 10.1080/09291010600902611

the phase response curve to light pulses (Underwood 1977, 1981, 1983). Seasonal variations in the influence of the pineal gland on the circadian system have been studied most notably in the ruin lizard, *Podarcis sicula*. Pinealectomy produces differential changes in the free running period of locomotor activity, depending upon the time of year (Innocenti et al. 1996; Bertolucci & Foà 1998; Foà & Bertolucci 2003). The seasonal changes in the influence of the pineal gland on the rest of the circadian system indicate that the pineal gland may mediate photoperiodic responses in some lizards (Hyde & Underwood 1993).

The fact that the lizard *Sceloporus torquatus* displays an annual reproductive cycle (Uribe-Alcocer et al. 1995) makes this a useful species for exploring the pineal seasonal influence on the circadian system. However, the role that the pineal gland plays in mediating seasonal rhythmicity is currently unknown. As such, the aim of this work is to observe if removing the pineal gland has differential effects on locomotor activity rhythms in *Sceloporus torquatus* during the summer and winter.

Methods

Animal maintenance and data acquisition

Adult lizards, *Sceloporus torquatus*, were collected from San Andrés Texcalyacác, Edo. de México and kept in the herpetarium facilities in the Facultad de Ciencias, Universidad National Autónoma de México. Animals were given water and live *Tenebrio molitor* larvae *ad libitum*. Lizards were kept individually in aquaria equipped with infrared light beams to detect locomotor activity. Light beams were located 2 cm above the bottom of the aquaria, in order to avoid detecting activity from the larvae. Beam breaks were summed in 10 min bins and the data stored to a PC by means of a data acquisition board (NAFRI disp. México D.F.). Aquaria were kept in light-tight, continuously ventilated wooden boxes and maintained at 23 ± 2°C in environmentally controlled rooms. A fluorescent lamp (150 lux) controlling the light conditions was located 30 cm above the glass aquarium and was controlled by a timer. Since the lizards require capturing live *T. molitor* larvae in order to eat, dim red light was provided by a small red bulb located at the top of the cabinet (30 cm distance, 1–2 lux), during the indicated as constant darkness (DD) or the dark phase of LD.

Data analysis

Data were collected every 10 minutes and stored in a PC until further analysis. Data were plotted in conventional actograms and the respective circadian periods were calculated using X^2 periodograms at 0–30 h intervals using the software DISPAC (Aguilar-Roblero et al. 1997). Locomotor activity was considered rhythmic when the periodogram had spikes above the confidence interval ($p < 0.05$). Average waveforms were obtained from a minimum of one week of data after stable rhythms were observed. The waveform constructed from the data from each experiment was fitted to the dominant period in order to obtain activity/rest ratios (α/ρ). Alpha (α) was considered to be the length of the activity phase and rho (ρ) the difference between free running period and alpha. Period lengths (τ) collected under the different conditions were analysed using a non-paired student T-test, by means of the software program Statistica (Stat-software, Jandel Scientific, San Rafael, CA, USA). Significant differences were considered when $p < 0.05$. All values are presented as means ± SE. Animals were kept in LD for one week prior to being placed in DD for a minimum of 10 days. Free running periods and waveforms were evaluated from stable rhythms.

Protocol

Pinealectomies were performed as previously described (Miranda-Anaya et al. 2000; Bartell et al. 2004). Briefly, all surgeries were performed on anaesthetized lizards by imbedding in crushed ice. Once immobile, a dental drill was used to bore a 3 mm hole in the skull behind the parietal eye and the pineal gland was removed using a pair of sterile fine forceps. During sham operations the pineal was not removed. The opening was packed with sterile wax and adhesive. Animals were allowed to recover while being warmed to room temperature on a thermal pad before being placed in the environmental test chamber. At the end of the experiment, lizards were sacrificed and the brain was extracted to visually confirm pinealectomy. Eleven lizards were field collected in November 2002; four were used for sham surgeries and seven for pinealectomy during the winter (January until March 2003). In order to compare to a different group during the summer, eight lizards were also collected and their activity was recorded during July until October 2003. Pinealectomy and sham surgeries were performed in the light; after recovery, the animals were kept in DRL for a minimum of two weeks before being transferred to LD for one additional week.

Results

In LD, activity is mainly distributed within the photic phase, although small bouts of activity were observed during darkness. The peak in activity occurred between midday and lights off. When animals were exposed to DD, the rhythm of activity free ran with a phase relationship similar to that observed in the previous LD conditions. Pinealectomy produced changes in the free running period of locomotor activity in all lizards from both groups (winter and summer). Each animal was considered as its own internal control, whereby pre- and post-surgery activity patterns were compared. Animals that received sham surgeries ($n=4$) did not show significant changes in the lengths of their free running circadian rhythms of activity (intact, $\tau = 23.9 \pm 0.2$ h, sham $\tau = 24.2 \pm 0.3$ h; $p > 0.05$). The actogram in Figure 1A shows the typical circadian rhythm from a lizard before and after sham surgery (arrow indicates the day of surgery). Figure 1B shows the entrained and free running circadian rhythm of a lizard tested during winter. After pinealectomy (Pin-X) was performed (second arrow), the animal was kept in the same conditions as those prior to surgery. A gradual change in period is observed, as well as a decrease in the amplitude of activity. In all pinealectomized animals, a change in the length of the free running circadian rhythm of locomotor activity was observed, as is shown in Figure 2. On the left, are the period lengths of the free running circadian rhythms from lizards collected in winter, and on the right are those obtained during summer. Pinealectomy produced changes in the free running period for all animals tested, however a tendency for larger changes was observed during winter (Figure 2 inner graph, winter $\tau = 0.6 \pm 0.17$; summer $\tau = 0.72 \pm 0.16$ h; $p > 0.05$).

Figure 3 shows the average ($\pm$SE) of the obtained values for α/ρ ratio in both DD and LD conditions. In DD (A) a reduction in alpha was observed during summer, and a tendency for it to increase during winter. However in LD, an increase in the amount of activity was observed after pinealectomy in LD conditions during summer (3B, star denotes $p < 0.05$).

Discussion

Pinealectomy does not eliminate the circadian rhythm of locomotor activity in *S. torquatus*. This finding is consistent with studies in other *Sceloporus* species (Underwood & Menaker

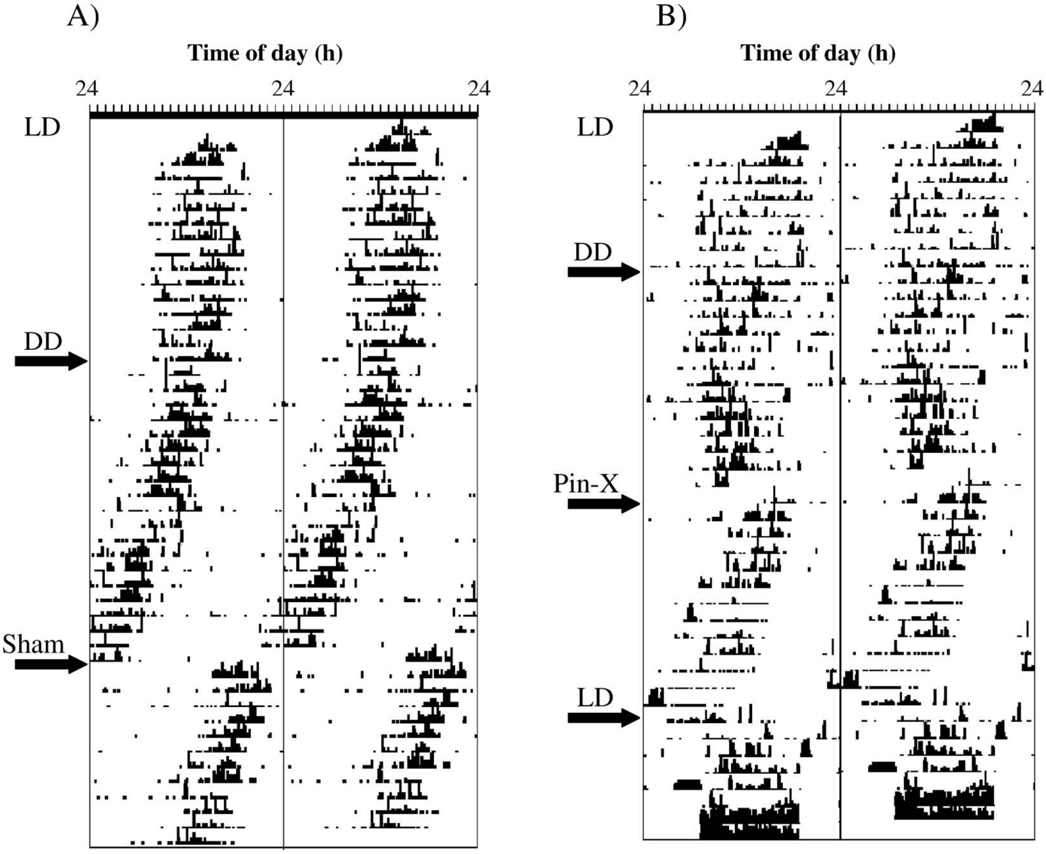

Figure 1. Locomotor activity of a sham operated (A) and a pinealectomized (B) lizard. Arrows indicate the change of condition. Pinealectomy produced a slight increase in the duration of alpha and the amplitude of activity was greatly reduced.

1970, 1976). In the present work we observed that removal of the pineal gland from *Sceloporus torquatus* always changes the free running period of locomotor activity. This has also been shown for other species of *Sceloporus* (Underwood 1977, 1981). The influences of the pineal gland on the free running period are likely mediated by the pineal hormone melatonin (Underwood 1990). It has been demonstrated that daily injections of melatonin can entrain locomotor activity rhythms in both intact and pinealectomized lizards (Underwood & Harles 1985; Miranda-Anaya et al. 2005). However, this effect does not seem to be differential when two opposite seasons are compared as observed in the European Ruin lizard *Podarcis sicula* (Foà & Bertolucci 2001). In *Sceloporus torquatus*, pinealectomy did not produce significant differential effects on the free running period of locomotor activity with regards to season. However, a tendency towards larger absolute changes in the free-running period during winter than in summer was observed (Figure 2). Taken together with the observed changes in α/ρ ratio, our findings do not preclude the lizard's ability to integrate seasonal information within the circadian system. Indeed, removing the pineal gland lengthens alpha during summer, but not in winter, and increases activity in LD while decreasing activity in DD. These results indicate that the strong effect of light is likely mediated by pineal melatonin in a similar way to that observed in other species of lizards (Hyde & Underwood 2000). The lizard

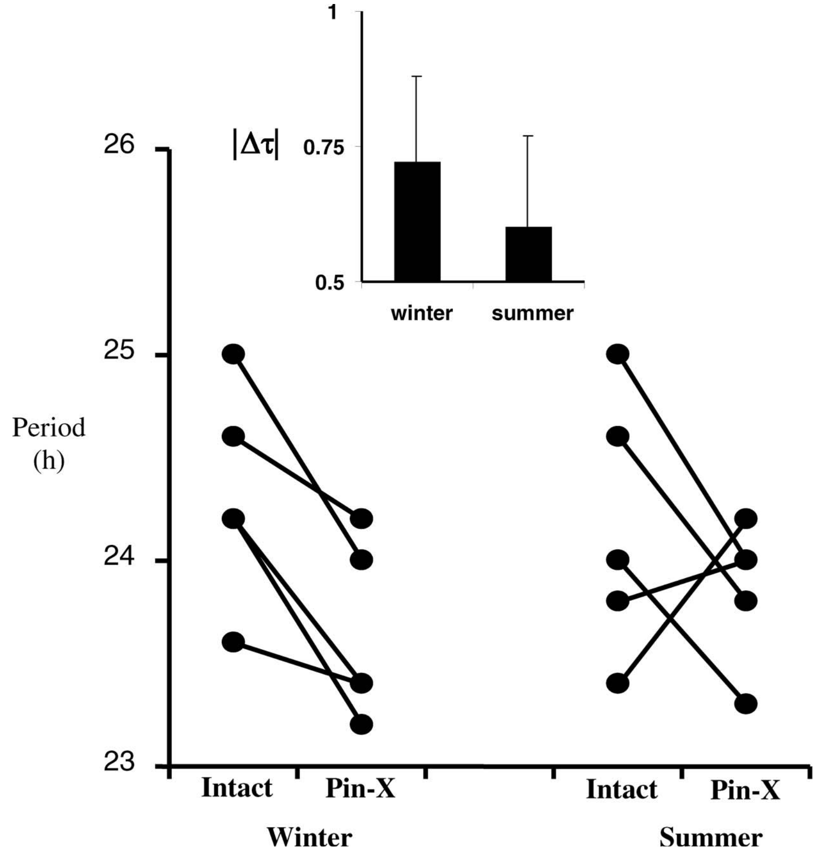

Figure 2. Changes in the free running period of locomotor activity rhythms, under constant conditions, during the winter and summer after pinealectomy. Values from individual animals are linked by a line. The upper graph shows the absolute difference in free running period (|Δτ|) after pinealectomy.

Sceloporus torquatus has an annual cycle of reproduction, with mating occurring during the autumn and offspring being born in the spring (Guillette et al. 1993; Uribe-Alcocer et al. 1995; Feria et al. 2001). This annual cycle may be due to the interactions of the circadian system with multiple environmental changes that occur throughout the seasons rather than any information transmitted by the pineal gland. For example, in the garter snake, *Thamnophis radix*, seasonal variations in the daily distribution of locomotor activity have been associated with seasonal changes in ambient temperature (Heckrotte 1960). As such, the seasonal differences observed after pinealectomy in other species may not translate to *Sceloporus torquatus,* and therefore the seasonal regulation of circadian behaviours by the pineal gland may be species-specific.

It has been suggested that photoperiodic changes may be mediated by photoreceptive structures outside the pineal gland (Foster & Soni 1998), most notably those located in the basal hypothalamus. However, different approximations can be made in order to see if the pineal gland is an important structure for photoperiodic responses in lizards, including studies on gonadal growth under artificial light and temperature conditions. Currently, the evidence demonstrating the involvement of the pineal gland in mediating seasonal changes in *Sceloporus* physiology is inconclusive and requires further examination.

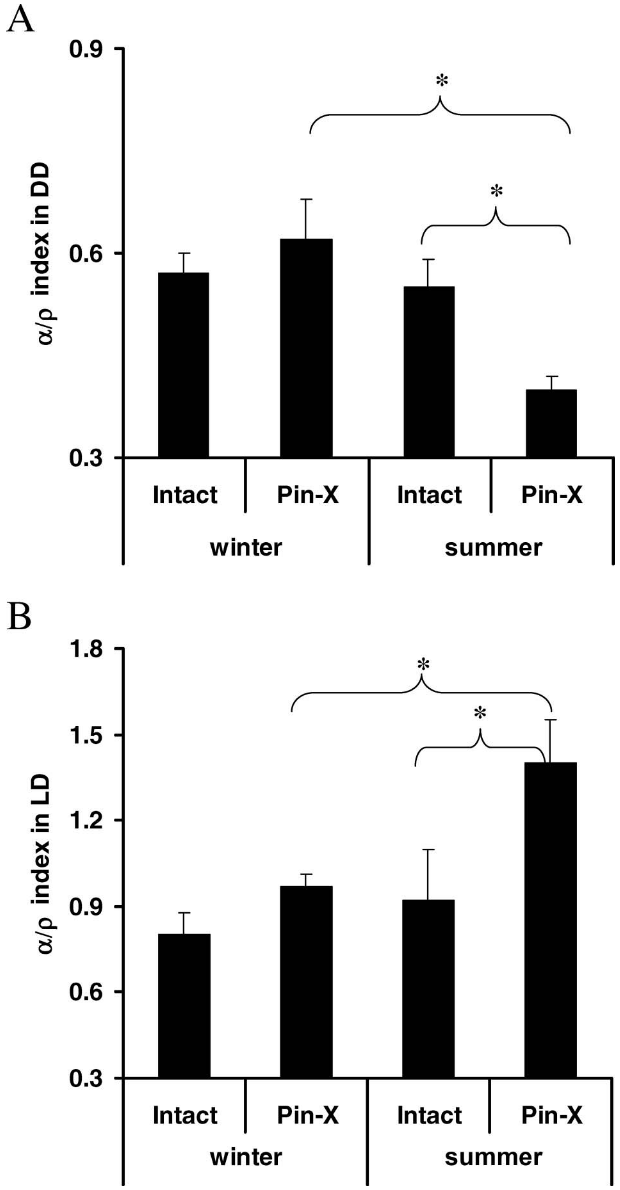

Figure 3. Changes in activity rest ratio (α/ρ) after pinealectomy (Pin-X) in animals tested during two different seasons. In (A), a reduction in α/ρ after pinealectomy was observed in animals held in DD in the summer. Statistical differences between groups (T-test, $p > 0.05$) are indicated by each asterisk.

Acknowledgments

We would like to thank Monica Salmerón and José León Pérez for assistance in obtaining the lizards. This work was funded by the Departmento de Biologia Celular, Facultad de Ciencias, UNAM.

References

Aguilar-Roblero R, Salazar-Juarez A, Rojas-Castaneda J, Escobar C, Cintra L. 1997. Organization of circadian rhythmicity and suprachiasmatic nuclei in malnourished rats. Am J Physiol 273:R1321 – 1331.

Bartell PA, Miranda-Anaya M, Menaker M. 2004. Period and phase control in a multi-oscillatory circadian system (*Iguana iguana*). J Biol Rhythms 19:47 – 57.

Bertolucci C, Foà A. 1998. Seasonality and role of SCN in entrainment of lizard circadian rhythms to daily melatonin injections. Am J Physiol Regul Integr Comp Physiol 274:1004 – 1014.

Feria M, Nieto-Montes A, Salgado IH. 2001. Diet and reproductive biology of the viviparous lizard *Sceloporus torquatus torquatus* (Squamata: Phrynosomatidae). J Herpetology 1:104 – 112.

Foà A, Bertolucci C. 2001. Temperature cycles induce a bimodal activity pattern in ruin lizards: Masking or clock controlled event? A seasonal problem. J Biol Rhythms 16:574 – 584.

Foà A, Bertolucci C. 2003. Toward a seasonal model of the circadian system: the case of ruin lizards. Frontiers in Bioscience 8:s236 – 242.

Foster RG, Soni BG. 1998. Extraretinal photoreceptors and their regulation of temporal physiology. Rev of Reprod 3:145 – 150.

Guillette LJ, Méndez-De la Cruz FR. 1993. The reproductive cycle of the viviparous Mexican lizard *Sceloporus torquatus*. J Herpetology 2:168 – 174.

Heckrotte C. 1960. The effects of environmental factors on the locomotory activity of the Plains garter snake (*Thamnophis radix radix*). PhD Thesis, University of Illinois.

Hyde LL, Underwood H. 1993. Effects of nightbreak, t-cycle, and resonance lighting schedules on the pineal melatonin rhythm of the lizard Anolis carolinensis. Correlations with the reproductive response. J Pineal Res 15:70 – 80.

Hyde LL, Underwood H. 2000. Effects of melatonin administration on the circadian activity rhythm of the lizard *Anolis carolinensis*. Physiol Behavior 7:183 – 192.

Innocenti A, Bertolucci C, Minutini L, Foà A. 1996. Seasonal variations of pineal involvement in the circadian organization of the ruin lizard *Podarcis sicula*. J Exp Biol 199:1189 – 1194.

Miranda-Anaya M, Bartell PA, Yamazaki S, Menaker M. 2000. Circadian rhythm of ERG in *Iguana iguana*: role of the pineal. J Biol Rhythms 15:163 – 171.

Miranda-Anaya M, Vásquez-Jiménez P, Barrera-Mera B. 2005. Effect of exogenous daily melatonin upon circadian locomotor activity under constant light conditions in the lizard Sceloporus torquatus. Program No. 766.19. Abstract. Washington, DC: Society for Neuroscience.

Tosini G, Bertolucci C, Foà A. 2001. The circadian system of reptiles: a multioscillatory and multiphotoreceptive system. Physiol Behavior 72:461 – 471.

Underwood H. 1977. Circadian organization in lizards: the role of the pineal organ. Science 195:587 – 589.

Underwood H. 1981. Circadian organization in the lizard, *Sceloporus occidentalis*: the effects of blinding, pinealectomy and melatonin. J Comp Physiol 141:537 – 547.

Underwood H. 1983. Circadian pacemakers in lizards: phase-response curves and effects of pinealectomy. Am J Physiol Regul Integr Comp Physiol 244:857 – 864.

Underwood H. 1990. The pineal and melatonin: regulators of circadian function in lower vertebrates. Experientia 46:120 – 128.

Underwood. 2001. Circadian Organization in non-mammalian vertebrates. In: Takahashi J, Turek F, Moore R, editors. Handbook of behavioral neurobiology, Vol 12 Circadian clocks. 1st ed. Amsterdam: Kluwer Academic Plenum Publishers. pp 111 – 140.

Underwood H, Harless M. 1985. Entrainment of the circadian activity rhythm of a lizard to melatonin injection. Physiol Behav 35:267 – 270.

Underwood H, Menaker M. 1970. Extraretinal light perception: entrainment of the biological clock controlling lizard locomotor activity. Science 70:190 – 193.

Underwood H, Menaker M. 1976. Extraretinal photoreception in lizards. Photochemistry and Photobiology 23: 27 – 243.

Uribe-Alcocer M, Méndez ME, Gonzalez JE, Guillettte LJ. 1995. Seasonal variation in ovarian histology of the viviparous lizard *Sceloporus torquatus torquatus*. J Morphol 226:103 – 119.