Western North American Naturalist 60(1), © 2000, pp. 98–100

REPRODUCTION IN THE TWIN-SPOTTED RATTLESNAKE, *CR0TALUS PRICEI* (SERPENTES: VIPERIDAE)

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Key words: reproduction, Crotalus pricei, *twin-spotted rattlesnake.*

The twin-spotted rattlesnake, *Crotalus pricei,* occurs in mountainous terrain of southeastern Arizona (Pinaleño, Graham, Dos Cabezas, Santa Rita, Huachuca, and Chiricahua Mountains) and south in the Sierra Madre Occidental of México to southern Durango from around 1220 to 3200 m (Stebbins 1985). Because there is limited information on reproduction in this species (Ernst 1992), the purpose of this note is to provide additional litter sizes and to present data on the timing of yolk deposition, ovulation, and testis cycle of *C. pricei.*

Data are presented from 31 sexually mature *C. pricei* (12 females, mean snout-vent length [SVL] = 400 mm ± 48 (*s*), range = 303–482 mm; 19 males, mean SVL = 433 mm ± 72 (*s*), range = 322–553 mm) and 1 litter of 7 neonates taken from the herpetology collections of Arizona State University (ASU), Natural History Museum of Los Angeles County (LACM), and University of Arizona (UAZ), Tucson (Appendix). One of the above females gave birth to 4 young and was not a museum specimen (D. Prival personal communication).

Counts were made of enlarged follicles (>6 mm length), oviductal eggs, or embryos. The left ovary was removed from females; the left testis, vas deferens, and part of the kidney were removed from males for histological examination. Tissues were embedded in paraffin and cut into sections at 5 µm. Slides were stained with Harris' hematoxylin followed by eosin counterstain. Testes slides were examined to determine stage of the male cycle; ovary slides were examined for presence of yolk deposition. Vasa deferentia were examined for sperm. Slides of kidney sexual segments were examined for secretory activity.

Not all tissues were available for histological examination due to damage or autolysis, but the following were examined: 9 ovaries, 19 testes, 18 kidneys, 14 vasa deferentia.

There is no previous information on the *C. pricei* testis cycle. Testicular histology was similar to that reported by Goldberg and Parker (1975) for 2 colubrid snakes, *Masticophis taeniatus* and *Pituophis catenifer,* and the viperid snake, *Agkistrodon piscivorus,* reported by Johnson et al. (1982). In recrudescent testes there was renewal of spermatogenic cells characterized by spermatogonial divisions; primary and secondary spermatocytes and spermatids may have been present. In spermiogenesis (which follows recrudescence), metamorphosing spermatids and mature sperm were present. None of the *C. pricei* males had regressed testes.

Males undergoing spermiogenesis were found June–October (Table 1). The smallest spermiogenic male measured 333 mm SVL, although 1 male with recrudescent testes that probably would have undergone spermiogenesis measured 322 mm SVL. Males smaller than this size (322 mm SVL) were excluded from the study to avoid the possibility of including immature specimens in analysis of the testis cycle. Testes in recrudescence were found June–August. Sperm were present in the vasa deferentia of 13/14 (93%) males including all those from June–September, indicating *C. pricei* has the potential for breeding throughout this period. Because 6/7 (86%) July males had recrudescent testes and 7/8 (88%) late summer–autumn males were undergoing spermiogenesis, the *C. pricei* testicular cycle may fit the aestival spermatogenesis "D" and postnuptial breeding pattern of Saint Girons (1982).

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TABLE 1. Monthly distribution of conditions in seasonal testicular cycle of *Crotalus pricei.* Values shown are numbers of males exhibiting each of the 2 conditions.

Month	*N*	Recrudescence	Spermiogenesis
June	4	2	2
July	7	6	1
August	3	1	2
September	4	0	4
October	1	0	1

In this pattern spermatogenesis occurs from June to October, with mating the following spring using sperm stored overwinter in the vasa deferentia, or during fall. Field observations of mating are needed to ascertain when *C. pricei* breeds.

Kidney sexual segments were enlarged and contained secretory granules in 16/18 (89%) kidneys examined from June to October: 6/7 (86%) males with recrudescent testes, 10/11 (91%) males with spermiogenic testes. Mating coincides with hypertrophy of the kidney sexual segment (Saint Girons 1982).

The smallest reproductively active female (UAZ 30952) measured 330 mm SVL (oviductal eggs). Three females (7 May, 11 June, 12 August; UAZ 20642, UAZ 33963, LACM 134040, respectively) were not undergoing yolk deposition (i.e., secondary vitellogenesis sensu Aldridge 1979). Two of the above females (7 May and 11 June) could have started yolk deposition and ovulated the following year. The 3rd (12 August) may have already given birth. Two females, 1 from 6 July (UAZ 42075) and the other from 27 September (LACM 75338) had started yolk deposition and may have ovulated the next year. One female (UAZ 35463) had enlarged follicles and likely would have ovulated the following year; it was collected 15 August and sacrificed 23 January (follicles >10 mm length). Four females had already ovulated (18 May, 7 June, 29 June, August, LACM 2964, UAZ 30952, ASU 7031, UAZ 47247, respectively) and likely would have given birth later that same year (Table 2). One female (LACM 104989) collected 7 July in Durango, México (SVL 375 mm), had a litter of 7 (mean SVL = 141 mm ± 4 *s*, range = 137–148 mm). It is not known whether the young were taken from the female or if she had given birth to them. One female gave birth 17 August to 4 young a few days after capture (D. Prival personal communication). Young are born July–August (Lowe et al. 1986).

The above data on the female reproductive cycle would lend support to the theory that *C. pricei* has a biennial reproductive cycle with females generally reproducing every other year as has been reported by Rahn (1942) for *Crotalus viridis* from southeastern Wyoming and Tinkle (1962) for *Crotalus atrox* from northwestern Texas.

Mean litter size for 7 *C. pricei* females (Table 2) was 5.1 ± 1.9 (*s*), range 3–8. This is within the 3–9 range reported by others for *C. pricei* (Kauffeld 1943a, 1943b, Stebbins 1954, Wright and Wright 1957, Keasey 1969, Klauber 1972, Armstrong and Murphy 1979, Van Devender and Lowe 1979, Mahaney 1997).

While useful information on reproductive biology can be gathered from histological examination of museum specimens, field studies on *C. pricei* are needed to reveal details of the reproductive cycle.

I thank Charles H. Lowe (University of Arizona), Robert L. Bezy (Natural History Museum of Los Angeles County), and Michael E. Douglas (Arizona State University) for permission

TABLE 2. Litter sizes for *Crotalus pricei.* Superscript letters indicate the following: c = captive born, e = embryos, f = enlarged follicles, o = oviductal eggs.

Date	SVL (mm)	Litter size	Locality	Source
18 May	400	4^{o}	Cochise Co., AZ	LACM 2964
7 June	330	4^{o}	Chihuahua, MX	UAZ 30952
29 June	482	8^{e}	Graham Co., AZ	ASU 7031
7 July	375	7^{c}	Durango, MX	LACM 104989
August	430	3^{e}	Chihuahua, MX	UAZ 47247
15 August	423	6^{f}	Chihuahua, MX	UAZ 35463
17 August	441	4^{c}	Cochise Co., AZ	D. Prival personal communication

to examine *Crotalus pricei.* David Prival (University of Arizona) provided information on 1 litter size. Cheryl Wong assisted with histology.

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Received 12 October 1998
Accepted 6 March 1999

APPENDIX

Specimens examined from herpetology collections at the Natural History Museum of Los Angeles County (LACM) and the University of Arizona (UAZ). **Arizona, Cochise County:** LACM 2964, 134040; UAZ 20642–20643, 27657–27658, 27662, 42075, 42080–42081, 42084–42086. **Graham County:** ASU 7031, 7047; UAZ 39586. **México, Chihuahua:** LACM 75338; UAZ 30952, 33963, 35080, 35234, 35463, 47247. **Coahuila:** UAZ 42556. **Durango:** LACM 104986–104996, 136979. **Nuevo Leon:** UAZ 46375.