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Research Note

Reproduction in the Baja California Collared Lizard, *Crotaphytus vestigium* (Squamata: Crotaphytidae)

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Crotaphytus vestigium, a rock-dwelling species of the peninsular ranges of Baja California, occurs along the northern slope of the San Jacinto Mountains, Riverside County, California, south to the southern margin of the volcanic Magdalena Plain in Baja California Sur (McGuire 1996). Published information on the reproduction of *C. vestigium* consists of brief accounts by Lemm (2006), Ivanyi, (2009) and field observations by McGuire (1996), Grismer (2002) and Stebbins (2003). The purpose of this paper is to examine the reproductive biology of *C. vestigium* from a histological analysis of gonadal material from museum specimens, an often used method, see for example, Goldberg (1974). Information on the reproductive cycle such as timing of spermiogenesis, number of egg clutches produced and period of vitellogenesis may not only be helpful in determining phylogenetic affinities, but also provides essential life history data for implementing conservation management strategies of lizard species.

We examined 61 *C. vestigium* consisting of 33 males (mean snout-vent length, SVL = 96.5 mm ± 12.3 SD, range: 72–116 mm and 28 females (mean SVL = 80.4 mm ± 8.6 SD, range: 54–93 mm) from Imperial, Riverside and San Diego Counties, California and Baja California and Baja California Sur, Mexico. Specimens were examined from the herpetology collections of the Natural History Museum of Los Angeles County (LACM), Museum of Vertebrate Zoology (MVZ), and San Diego Society of Natural History (SDSNH) (Appendix I). Lizards were collected 1934–1997. Histology slides were deposited at LACM, MVZ and SDSNH.

The left testis was removed from males and the left ovary was removed from females for histological examination (Presnell and Schreibman 1997). Enlarged ovarian follicles (> 5 mm) and/or oviductal eggs were counted. Tissues were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin followed by eosin counterstain. Ovary slides were examined for yolk deposition or corpora lutea. Testis slides were examined to ascertain the stage of the testicular cycle present. Mean SVL of male and female *C. vestigium* were compared using an unpaired *t*-test (Instat vers. 3.0b, Graphpad Software, San Diego, CA).

The mean male SVL of *C. vestigium* significantly exceeded that of females (unpaired *t* test, $t = 5.82$, $df = 59$, $P < 0.0001$). Monthly stages in the testicular cycle of *C. vestigium* were shown in Table 1. Three stages were present: (1) Regression, the germinal epithelium was reduced to 1–3 cell layers in thickness and consists of spermatogonia and Sertoli cells; (2) Recrudescence, a proliferation of germ cells for the next period of sperm formation was underway. In early recrudescence, primary spermatocytes predominate,

Table 1. Monthly stages in the testicular cycle of *Crotaphytus vestigium.*

Month	*n*	Regression	Recrudescence	Spermiogenesis
March	6	1	4	1
April	6	0	2	4
May	8	0	2	6
June	2	0	0	2
July	6	0	0	6
August	4	4	0	0
November	1	1	0	0

whereas in late recrudescence, secondary spermatocytes and spermatids were most abundant; (3) Spermiogenesis, lumina of the seminiferous tubules were lined by clusters of sperm or clusters of metamorphosing spermatids. The smallest reproductively active male (LACM 63168) with spermiogenesis in progress measured 73 mm SVL and occurred in July. One male collected in April (LACM 138523) measured 72 mm SVL and exhibited testicular recrudescence. It was not known when this individual would have commenced spermiogenesis.

The testicular cycle of *C. vestigium* was typical of other lizards from western North America that undergo spermiogenesis beginning in spring and terminate in summer (see Goldberg 1974, 1975, 1977, 1983). The congener *Crotaphytus collaris* follows a testicular cycle similar to that of *C. vestigium*. However, in west-central Texas, reproductive activity in *C. collaris* was observed from April into July (Ballinger and Hipp 1985). The onset of sperm production was delayed until early May in Arkansas (Trauth 1979). This suggests some geographic variation in the reproductive cycle of *C. collaris*. It was not known whether there was geographic variation in the reproductive cycle of *C. vestigium*, although one individual (Table 1) in spermiogenesis (MVZ 73568) in March was from the southern part of its range in Baja California Sur, Mexico. Lemm (2006) reported breeding of *C. vestigium* in May and June, although data were lacking to support this claim. However, our results suggested breeding may commence in April. This was corroborated by McGuire (1996), Grismer (2002) and Stebbins (2003), who reported *C. vestigium* from Baja California Sur with breeding coloration in April.

Four stages were present in the ovarian cycle of *C. vestigium*: (1) no yolk deposition (quiescent); (2) early yolk deposition with basophilic granules present; (3) enlarged preovulatory follicles; (4) oviductal eggs. Monthly changes in the ovarian cycle were presented in Table 2. The smallest reproductively active *C. vestigium* female (LACM 63169) measured 75 mm SVL and was undergoing yolk deposition. The maturity of two females (LACM 4000, SVL = 67 mm and SDSNH 17667, SVL = 54 mm) was doubtful, and were excluded from Table 2. Mean clutch size (enlarged ovarian follicles > 5 mm or oviductal eggs) for three females was 2.67 ± 1.2 SD, range: 2–4. One clutch from June (SDSNH 60111) was damaged and could not be counted (Table 2). Lemm (2006) reported 1–2 clutches of 8 or more eggs, although we know of no report in the literature documenting multiple clutching for *C. vestigium*. Ivanyi (2009) reported clutches of 3–8 eggs with breeding lasting until late summer. Our observation of two eggs was an unreported minimum clutch size for *C. vestigium*.

It appeared that the period of female reproductive activity encompasses spring and summer (Table 2). Since all seven females from May had quiescent ovaries (Table 2) it was possible yolk deposition was delayed in some females or not all females reproduced

Table 2. Monthly stages in the ovarian cycle of *Crotaphytus vestigium*. A female in June contained damaged oviductal eggs which were not counted.

Month	*n*	Quiescent	Early yolk deposition	Enlarged follicles > 5 mm	Oviductal eggs
March	1	1	0	0	0
April	2	0	2	0	0
May	7	7	0	0	0
June	2	0	0	1	1*
July	11	5	4	1	1
September	2	2	0	0	0
December	1	1	0	0	0

each year. Two females from Baja California (LACM 16993) and Riverside County (LACM 52889) contained vitellogenic follicles in April. Thus, there was no indication of yolk deposition commencing earlier in the south, although our female sample size was too small to clarify this issue. Four females from July undergoing early yolk deposition (Table 2) also raised questions. We had no females from the month of August, so it was not possible to conclude if late season egg clutches were produced. As in other North American lizards (Goldberg 1973, 1975), vitellogenic follicles occurring late in the breeding season might typically undergo atresia and yolk reabsorption. On the other hand, Grismer (2002) reported a female with gravid coloration in early October, just east of Canipolé, Baja California Sur which suggests eggs were produced late in the year.

Considering the ovarian cycle of the congener *C. collaris,* females from Arkansas and Utah ceased reproduction at the end of June (Trauth 1978, Andre and MacMahon 1980). In the arid regions of southern California and Baja California, the ovarian cycle of *C. vestigium*, was of longer duration and extends into August and perhaps October (Grismer 2002). This difference tended to support the separation of *C. collaris* and *C. vestigium* into separate species.

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Appendix I

Crotaphytus vestigium examined from the Natural History Museum of Los Angeles County (LACM), Museum of Vertebrate Zoology (MVZ), and San Diego Society of Natural History (SDSNH).

LACM Baja California 4000, 16993, 16995, 16996, 63176, 94681, 138523, Baja California Sur, 16994, 63167-63171, 63173-63175, 63177, 63178, California, Imperial County, 146603, Riverside County 16873-16875, 52889, 52890, 94625, 94627-94629, 122043; MVZ Baja California, 50016, 51140, 140754, 140755, Baja California Sur 73568; SDSNH Baja California, 17052, 17667, 19788-19792, 26754, 37815, 41612, Baja California Sur, California, 30107-30111, Imperial County, 60110, 60111, 60216, 62822, 62823, Riverside County 20699, San Diego County 11088, 11951, 13250, 29698, 40353, 58391.