

## Notes on reproduction of the Sierra Nevada yellow-legged frog from California

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Sierra Nevada yellow-legged frog (*Rana sierrae* Camp, 1917) is known from the northern and central Sierra Nevada of California (Vredenburg et al. 2007) where it frequents elevations of ca. 2,500-3,500 m (Dodd 2013). It is mainly diurnal and breeds between late May to July (Stebbins and McGinnis 2012). It is listed as threatened by the California Fish and Game Commission under the California Endangered Species Act (CNDDDB 2019), endangered by the U.S. Fish and Wildlife Service (ECOS 2019) and imperiled by NatureServe Explorer (2019). The reproductive biology of a species is an important part of its natural history and such information is crucial in conservation plans (see Bury 2006). In this paper I add information on reproduction of *R. sierrae* from a histological examination of gonadal tissues. The use of museum collections for obtaining reproductive data avoids euthanizing specimens and obviates the need for a collecting permit by state and federal authorities.

A sample of 32 *R. sierrae* from California collected 1948 to 1972 (Appendix) consisting of 6 adult males (mean snout-vent length, SVL = 60.3 mm  $\pm$  3.3 SD, range = 54–63 mm), 22 adult females (mean SVL = 70.0 mm  $\pm$  7.1 SD, range = 60–86 mm), 1 unsexed subadult (SVL = 38 mm) and 3 subadult females (SVLs 46, 50, 58 mm) from the herpetology collection of the Natural History Museum of Los Angeles County (LACM), Los Angeles, California, USA was examined. I examined frogs from Madera and Mono counties (Clade 2) and Inyo and Mariposa counties (Clade 3) as designated by Vredenburg et al. (2007). I tested for differences between adult male and female SVLs using an unpaired t-test (Instat, vers. 3.0b, Graphpad Software, San Diego, CA, USA).

A small incision was made in the lower part of the abdomen, and the left testis was removed from males and a piece of the left ovary from females. I embedded gonads in paraffin, and sections were cut at 5  $\mu$ m and stained with Harris hematoxylin followed by eosin counterstain (Presnell and Schreiber 1997). Histology slides were deposited at LACM.

The testicular morphology of *R. sierrae* is similar to that of previously studied anurans as detailed in Ogielska and Bartmanska (2009a). Within the seminiferous tubules, spermatogenesis occurs in cysts which are closed until the late spermatid stage is reached; cysts then open and differentiating sperm reach the lumina of the seminiferous tubules (Ogielska and Bartmanska 2009a). A ring of germinal cysts is located on the inner periphery of each

seminiferous tubule. All 6 *R. sierrae* males in my sample were undergoing spermiogenesis. By month these were: June (N = 1), July (N = 1), August (N = 4). The smallest mature male *R. sierrae* (spermiogenesis) measured 54 mm SVL and was from June (LACM 13565).

The mean SVL of *R. sierrae* females was significantly larger than that of males ( $t = 3.2$ ,  $df = 26$ ,  $P = 0.004$ ). The ovaries of *R. sierrae* are typical of other anurans in being paired organs lying on the ventral sides of the kidneys. In adults the ovaries are filled with diplotene oocytes in various stages of development (Ogielska and Bartmanska 2009b). Mature oocytes are filled with yolk droplets; the surrounding layer of follicular cells is thinly stretched. Monthly stages in the ovarian cycle of *R. sierrae* are in Table 1. Two stages were present (1) "Ready to spawn" in which mature oocytes predominated; (2) "Not in spawning condition" in which previtellogenic oocytes predominated. There were nine females in the "not in spawning condition" in Table 1. Two of them, LACM 91271 (June) and LACM 67392 (July) contained early diplotene oocytes and partial yolk filled oocytes. The yolking oocytes in these two females were similar to Secondary Growth Stage 5 "progressive accumulation of yolk platelets" as reported by Uribe Aranzábal (2011). It is not known when or if these two females would have reached spawning condition. The smallest mature female (LACM 13568) measured 63 mm SVL, was from July, and contained mature yolk-filled oocytes. Three slightly smaller nonreproductive females, two of which measured 60 mm in SVL (LACM 13603, 13610) and a third LACM 67392, (SVL = 62 mm), the only one to contain partially yolking oocytes (see above), were arbitrarily considered as adults. Three yet smaller nonreproductive females (LACM 13613, 13614, 1741) SVLs = 46, 58, 50 mm, respectively, were considered to be subadults. One smaller unsexed *R. sierrae*, (LACM 13572, 38 SVL mm) was also considered as a subadult. I cannot speculate as to when any of these small *R. sierrae* would have attained adult size.

Atretic follicles were observed in 13 of 22 (59%) of my mature female sample. Atresia is a widespread process occurring in the ovaries of all vertebrates (Uribe Aranzábal 2009). It is common in the amphibian ovary (Saidapur 1978) and is the spontaneous digestion of a diplotene oocyte by its own hypertrophied and phagocytic granulosa cells which invade the follicle and eventually degenerate after accumulating dark pigment (Ogielska and Bartmanska 2009b). See Saidapur and Nadkarni (1973) and Ogielska et al. (2010) for a detailed description of stages of atresia in the frog ovary. Atresia plays an important role in fecundity by influencing numbers of ovulated oocytes (Uribe Aranzábal 2011).

In conclusion, my data on females indicates *R. sierrae* is in spawning condition during July and August. Whether *R. sierrae* females complete spawning in late summer or enter hibernation while containing mature oocytes warrants additional study.

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## APPENDIX

Thirty-two *R. sierrae* from California (by county) examined from the herpetology collection of the Natural History Museum of Los Angeles County (LACM), Los Angeles, California, USA.

**Inyo:** LACM 67391–67393; **Madera:** 1966–1973, 13568, 13572, 26707, 91271, 91272; **Mariposa:** LACM 13599–13606, 13609–13614; **Mono:** 1741, 13565.

**Table 1.** Two monthly stages in the spawning cycle of 22 adult female *R. sierrae* from California.

Month	N	Ready to spawn	Not in spawning condition
June	2	0	2
July	4	3	1
August	16	10	6