

Notes on reproduction of Cascades frogs from California

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Cascades frogs (*Rana cascadae* Slater, 1939) occur in three disjunct areas: (1) the Olympic Mountains of Washington, (2) the Cascades Mountains of Washington and Oregon and (3) the Klamath-Siskiyou Mountains in northern California (Dodd 2013). *Rana cascadae* is now nearly extinct from the northern end of the Sierra Nevada (Lassen, Plumas, Shasta and Tehama counties, California; Thomson et al. 2016) and is listed as vulnerable by NatureServe Explorer (2019). Factors responsible for the disappearance of *R. cascadae* in the southern part of its range are discussed in Fellers and Drost (1993).

Information on reproduction of *R. cascadae* is limited. *Rana cascadae* males appear at breeding sites as ice melts; in Oregon, breeding begins in March and April (Briggs 1987). Slater (1939) reported spawning in Washington occurred from May 20 to July 10, but dates vary depending on snowfall.

In this paper, I present data from a histological examination of *R. cascadae* gonadal material from Plumas County, California. Utilization of museum collections for obtaining reproductive data avoids euthanizing specimens and eliminates the need for collecting permits from state and federal authorities. In the case of the nearly extinct *R. cascadae* in California, histological information on timing of events in its reproductive cycle will prove useful in subsequent attempts to reestablish this species in its former range.

I examined a sample of 36 *R. cascadae* collected 1954 to 1972 in Plumas County (39.9927°N, 120.8039°W) California consisting of 11 adult males (mean snout-vent length, SVL = 43.9 mm ± 6.9 SD, range = 28–53 mm), 16 adult females (mean SVL = 56.4 mm ± 6.9 SD, range = 45–70 mm), and 9 unsexed subadults (mean SVL = 27.2 mm ± 9.1 SD, range = 17–40 mm) from the herpetology collection of the Natural History Museum of Los Angeles County (LACM), Los Angeles, California, USA (Appendix). An unpaired *t*-test was used to test for differences between adult male and female SVLs (Instat, vers. 3.0b, Graphpad Software, San Diego, CA).

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. Gonads were embedded in paraffin, and sections were cut at 5 µm and stained with Harris hematoxylin followed by eosin counterstain (Presnell and Schreiber 1997). Histology slides were deposited at LACM.

The testicular morphology of *R. cascadae* is similar to that of other anurans as described in Ogielska and Bartmanska (2009a). Within the seminiferous tubules, spermiogenesis 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). Six of seven *R. cascadae* males from August and all four from September exhibited spermiogenesis (sperm formation) in which sperm cysts were open and clusters of sperm were present in the lumina of the seminiferous tubules. A ring of germinal cysts was located on the inner periphery of each seminiferous tubule. The seminiferous tubules of one August *R. cascadae* male (LACM 76645, SVL = 38 mm) contained germinal cysts, but no sperm. The smallest male *R. cascadae* in my sample (LACM 76643) measured 28 mm SVL and contained a few small sperm clusters in the lumina of most of the seminiferous tubules. Clusters of spermatids were present in those seminiferous tubules that lacked sperm. On the basis of sperm being present in most seminiferous tubules, I considered this *R. cascadae* to be an adult, although it is not known if it would have joined the breeding population. Wright and Wright (1970) reported adult males of *R. cascadae* measured 50–58 mm in body length. My smallest male to exhibit full spermiogenesis (lumina of seminiferous tubules lined with sperm or clusters of metamorphosing spermatids) measured 40 mm SVL, was from August (LACM 76649), and is ten mm smaller than the minimum size for *R. cascade* male maturity in Wright and Wright (1970).

The mean SVL of *R. cascadae* females was significantly larger than that of males ($t = 4.6$, $df = 25$, $P < 0.001$). The ovarian morphology of *R. cascadae* is similar to that of other anurans in being paired organs situated on the ventral sides of the kidneys, and in adults, ovaries are filled with diplotene oocytes in various stages of development (Ogielska and Bartmanska 2009b). Mature oocytes are filled with yolk droplets and the layer of surrounding follicular cells is thinly stretched. Two stages were present in the spawning cycle (Table 1): (Stage 1) “Ready to spawn” in which mature oocytes predominate and (Stage 2) “Not in spawning condition” in which previtellogenic oocytes predominate. All seven *R. cascadae* females from June, one from August, and seven of eight from September exhibited Stage 1 “Ready to Spawn” ovaries. One September female exhibited Stage 2 “Not in Spawning Condition” (SVL = 48 mm, LACM 76621) and contained previtellogenic oocytes. It may have spawned earlier in the year. The smallest mature *R. cascadae* female (ready to spawn) measured 48 mm SVL (LACM 76632) and was from September. Wright and Wright (1970) reported adult females of *R. cascadae* measured 52–74 mm in body size.

Varying amounts of atresia were noted in eight of fifteen (53%) *R. cascadae* spawning females (Table 1). Atresia is a widespread process occurring in the ovaries of all vertebrates (Uribe Aranzábal 2009) and is common in the amphibian ovary (Saidapur 1978). It 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

Table 1. Two monthly stages in the spawning cycle of 16 adult female cascade frogs.

Month	N	(1) Ready to spawn	(2) Not in spawning condition
June	7	7	0
August	1	1	0
September	8	7	1

et al. (2010) for a detailed description of the stages of follicular atresia in the frog ovary. Atresia plays an important role in fecundity by influencing numbers of ovulated oocytes (Uribe Aranzábal 2011).

Regarding my sample of 9 juveniles, I am unable to ascertain when they would have reached adult size. However, according to Wright and Wright (1970) newly metamorphosed *R. cascadae* measured 20–24 mm. Five of my *R. cascadae* juveniles were in the 17–23 mm range and were likely young of the year. Four of these were from August and one from September.

The absence of female *R. cascadae* samples from early spring did not allow a complete description of monthly stages in the ovarian cycle. However, previous work (Slater 1939, Briggs 1987) indicates reproduction commences shortly after *R. cascadae* emerge from winter inactivity. Regarding the *R. cascadae* females in spawning condition from later in the year, August and September (Table 1), it is plausible they would have kept their ripe eggs until spring before spawning. This appears to be the case for *R. boylii* from California as reported by Goldberg (2019) in which females from autumn with mature oocytes apparently delay spawning until spring (Zweifel 1955). The retention of mature oocytes over winter allows *R. cascadae* to spawn soon after emergence from hibernation and avoids delay from needing to undergo a period of yolk deposition.

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APPENDIX

Thirty-six *R. cascadae* examined from Plumas County, California borrowed from the herpetology collection of the Natural History Museum of Los Angeles County, Los Angeles, California, USA.

LACM: 13408, 13409–13411, 13413, 13414, 13419, 13422, 13426, 14915, 74422, 76617–76624, 76626, 76627, 76629, 76632, 76634, 76638, 76641–76643, 76645–76652.