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Notes on Reproduction of the Boreal Chorus Frog, *Pseudacris maculata* (Anura: Hylidae), from Colorado

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Pseudacris maculata (Agassiz, 1850) (Fig. 1) occurs from Northwest Territories, Canada and Wisconsin south through Montana, Idaho, southeastern Utah to northeastern Arizona, northern New Mexico, northern Oklahoma, northwestern Arkansas, Missouri, Illinois and western Indiana with disjunct populations in southern Ontario and southern Quebec (Canada), northern New York and northwestern Vermont (Frost 2021). *Pseudacris maculata* has previously been called *P. nigrita maculata*, *P. triseriata maculata*, *P. triseriata*, and *Hyla (Pseudacris) maculata* in the literature (Frost 2021). It is a cold-tolerant terrestrial frog that can withstand freezing temperatures (Murphy 2019). Reproduction of *P. maculata* occurs from spring into early summer, but varies depending on altitude and latitude; there may be variation in initiation of breeding due to environmental conditions and the numbers of breeding adults may fluctuate from year to year (Dodd 2013). *Pseudacris maculata* are among the earliest of spring breeders (Green et al. 2013). In Alberta, males of *P. maculata* begin calling before the snow melts (Russell and Bauer 2000). Data from the Colorado Plateau of neighboring Arizona indicate *P. maculata* may commence reproduction in late March into April (Bezy et al. 2004). Eggs are deposited in small clusters attached to vegetation (Russell and Bauer 2000). Corn (1980) observed *P. maculata* (as *P. triseriata*) breeding from 21 April to 4 June in Larimer County, Colorado. In Colorado *P. maculata* may remain active after the first snowfall (Young 2011). In this paper I present data from a histological examination of *P. maculata* gonads from Colorado. Utilization of museum collections for obtaining reproductive data avoids removing additional animals from the wild.

A sample of 21 *P. maculata* from Colorado (Appendix) collected 1957 to 1992 consisting of 14 adult males (mean snout-vent length, SVL = 27.9 mm \pm 3.2 SD, range = 23-33 mm) and 7 adult females (mean SVL = 28.3 mm \pm 2.4 SD, range = 25-33 mm) was examined from the vertebrate collections of the University of Colorado Museum of Natural History (UCM), University of Colorado Boulder, Colorado, 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. Gonads were embedded in paraffin, sections were cut at 5 μ m and stained with Harris hematoxylin followed by eosin counterstain (Presnell and Schreiber 1997). Histology slides were deposited at UCM. An unpaired *t*-test was used to test for differences between male and female SVLs (InStat, vers. 3.0b, Graphpad Software, San Diego, CA).



Fig. 1. Boreal Chorus Frog (*Pseudacris maculata*), Edmonton, Canada. Photo Public Domain.

There was no significant difference between mean SVL of adult males versus adult females of *P. maculata* ($t = 0.2592$, $df = 19$, $P = 0.7983$). The testicular morphology of *P. maculata* 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). A tangled mass of spermatozoa or open sperm cysts was observed in the lumen of each seminiferous tubule. A ring of germinal cysts is located on the inner periphery of each seminiferous tubule. There was no discernible testicular cycle in my monthly samples of *P. maculata* males as all exhibited spermiogenesis (sperm formation): April ($n = 3$), April-May ($n = 2$), May ($n = 3$), June ($n = 3$), July ($n = 1$), August ($n = 2$). The two smallest sexually mature males of *P. maculata* both measured 23 mm SVL (UCM 11170 from June and UCM 34516 from April). Wright and Wright (1933) reported adult males of *P. maculata* (as *P. nigrita triseriata*) measured 21-32 mm,

The ovaries of *P. maculata* are similar to those 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 layer of surrounding follicular cells is thinly stretched. Six of my seven *P. maculata* females were in spawning condition in which mature oocytes predominated. They came from the following months: May ($n = 4$), August ($n = 1$), September ($n = 1$). The ovary from the remaining female, from July (25 mm SVL, UCM 34584), was not reproductively active, and contained previtellogenic oocytes. The smallest mature *P. maculata* female

Pseudacris maculata (Agassiz, 1850) (Fig. 1) occurs from Northwest Territories, Canada and Wisconsin south through Montana, Idaho, southeastern Utah to northeastern Arizona, northern New Mexico, northern Oklahoma, northwestern Arkansas, Missouri, Illinois and western Indiana with disjunct populations in southern Ontario and southern Quebec (Canada), northern New York and northwestern Vermont.

(spawning condition) in my sample measured 27 mm SVL (UCM 9954) and was from May. Wright and Wright (1933) reported adult females of *P. maculata* (as *P. nigrita triseriata*) ranged from 20.0 to 37.5 mm. My two *P. maculata* females, (August UCM 55588; September UCM 49466), each in spawning condition suggests some reproduction may occur in Colorado during late summer. August spawning has previously been reported for *P. maculata* in Arizona, Nebraska and New Mexico (Table 1).

Three of my six (50%) *P. maculata* females in spawning condition contained atretic follicles. Atresia is a widespread process occurring in the ovaries of all vertebrates (Uribe Aranzabal 2009). Atresia 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 follicular atresia in the frog ovary. Atresia plays an important role in fecundity by influencing numbers of ovulated oocytes (Uribe Aranzabal 2011). Incidences of follicular atresia increase late in the reproductive period (Saidapur 1978). Saved energy will be presumably utilized during a subsequent reproduction.

No postovulatory follicles (evidence of a recent

spawning) were noted in my sample of *P. maculata* females. Postovulatory follicles form when the ruptured follicle collapses after ovulation; the follicular lumen disappears and proliferating granulosa cells are surrounded by a fibrous capsule (Redshaw 1972). Postovulatory follicles are short-lived in most anuran species and are resorbed after a few weeks (Redshaw 1972). Their absence may be the result of my small sample of *P. maculata* females.

In conclusion, my data indicate that in Colorado, the *P. maculata* reproductive cycle commences in spring. Based on the presence of one female *P. maculata* each in spawning condition from August and September, and August males containing sperm, reproduction in Colorado may continue into late summer. Months of breeding for *Pseudacris maculata* from different states and Canadian Provinces are shown in Table 1.

Acknowledgments—I thank Emily M. Braker (UCM) for permission to examine *P. maculata* and for facilitating this loan (V-220).

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Table 1. Months of breeding for *Pseudacris maculata* from different states.

State	Breeding Period	Source	Reported as
Alberta	April to June	Russell and Bauer 2000	<i>P. maculata</i>
Arizona	February to August	Holycross et al. 2021	<i>P. maculata</i>
Arizona	March-April	Bezy et al. 2004	<i>P. triseriata complex</i>
British Columbia	early May through June	Matsuda et al. 2006	<i>P. maculata</i>
Colorado	April to July	Hammerson 1999	<i>P. triseriata</i>
Idaho	April to June	Groves 1989	<i>P. triseriata</i>
Illinois	Mid February through May	Phillips et al. 1999	<i>P. triseriata</i>
Iowa	March to May	LeClere 2013	<i>P. maculata</i>
Kansas	chorus February through August	Collins et al. 2010	<i>P. maculata</i>
Manitoba	April to late June	Preston 1982	<i>P. triseriata maculata</i>
Minnesota	March-early May	Moriarty and Hall 2014	<i>P. maculata</i>
Missouri	February-April	Johnson 2000	<i>P. triseriata triseriata</i>
Montana	May to July	Black 1970	<i>P. triseriata maculata</i>
Montana	April to June	Werner et al. 2004	<i>P. maculata</i>
Nebraska	start early March	Fogell 2010	<i>P. maculata</i>
Nebraska	early March to late August	Ballinger et al. 2010	<i>P. triseriata</i>
New Mexico	spring to August	Degenhardt et al. 1996	<i>P. triseriata</i>
North Dakota	April to June	Wheeler and Wheeler 1966	<i>P. nigrita</i>
Oklahoma	February to June	Sievert and Sievert 2011	<i>P. maculata</i>
Ontario	early spring	Christie 1997	<i>P. triseriata triseriata</i>
South Dakota	April to late May	Kiesow 2006	<i>P. maculata</i>
Quebec	May-June	Desroches and Rodrigue 2004	<i>P. maculata</i>
Yellowstone & Grand Teton	late March-early April	Koch and Peterson 1995	<i>P. triseriata maculata</i>
Wisconsin	March to May-June	Vogt 1981	<i>P. triseriata triseriata</i>
Wyoming	late March to early April	Baxter and Stone 1980	<i>P. triseriata maculata</i>
Wyoming	calling in early spring	Lewis 2011	<i>P. maculata</i>
No specific locality	March 20 to May 20	Wright and Wright 1933	<i>P. nigrita triseriata</i>

No postovulatory follicles (evidence of a recent spawning) were noted in my sample of *P. maculata* females. Postovulatory follicles form when the ruptured follicle collapses after ovulation; the follicular lumen disappears and proliferating granulosa cells are surrounded by a fibrous capsule (Redshaw 1972).

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Appendix: Twenty-one *P. maculata* from Colorado examined (by county) from the herpetology collection of University of Colorado Museum of Natural History (UCM), The University of Colorado, Boulder, Colorado USA. **Adams:** UCM 19612, 49466; **Boulder:** UCM 11108, 11113, 21956, 34516; **Delta:** UCM 11817; **Grand:** UCM 11170, 11172, 55588; **Jackson:** UCM 34675; **Jefferson:** 42949, 47530; **Larimer:** UCM 34788, 34813; **Mesa:** UCM 34584, 34585; **Moffat:** UCM 56577; **Montezuma:** UCM 25675; **Weld:** 9952, 9954.

NATURAL HISTORY NOTE

Highest-known elevation documentation of a Gila monster (*Heloderma suspectum*) in the Rincon Mountains, southern Arizona

Daniel Beckman (danielbeckman@gmail.com), **Nicole Gonzalez** (nicole_gonzalez@nps.gov), and **Don E. Swann** (Don_Swann@nps.gov), Saguaro National Park, 3693 South Old Spanish Trail, Tucson, Arizona, USA, AZ

The Gila Monster (*Heloderma suspectum*) occurs throughout the Sonoran Desert. It is typically observed in low elevation desert areas, but ranges into semi-desert grasslands and oak woodland communities. The highest validated observation is 1,743 m (Beck 2005), although they have been reported to occur to 1,950 m (Degenhardt et al. 1996).

Two of us (DB and NG) observed and photographed (Fig. 1) an adult Gila Monster in the Rincon Mountains at an elevation of 2,089 m at 11:17 AM on 5 August 2019. Coordinates for the location were 32.194384°N, 110.564834°W; NAD 83. We confirmed the location and elevation with a Trimble Juno 5 GPS unit, and elevation recorded on the unit was consistent with the elevation based on later mapping of the exact location using ArcGIS software.

The habitat was oak woodland, with common plants including *Quercus hypoleucoides* (silver-leaf oak), *Quercus rugosa* (netleaf oak), *Pinus discolor* (piñon pine), *Juniperus deppeana* (alligator juniper), and *Garrya wrightii* (Wright's silktassel). The aspect of the area was generally southwest. The Rincon Mountains are a northern "Sky Island" mountain range in south-central Arizona near Tucson. An adult *Gopherus morafkai* (Sonoran Desert Tortoise) was photographed in the near vicinity in 1999, an elevational record for that species (Aslan et al. 2003).

Saguaro National Park has an on-going "community science" program where visitors are encouraged to take photos of Gila Monsters from a safe distance without disturbing the animal, and send the photos to gilamonsterresearch@gmail.com. Individual animals can be identified by the color pattern of their scales, unique to each individual, which provides data on longevity, home range size, and other aspects of the natural history of this interesting lizard. As of 2020 the park had received more than 660 photographs and identified about 500 different Gila Monsters since 2005. We are continuing to seek photographs of Gila Monsters in the park or within one half-mile of the park boundary only. For more information on this project, see Gila Monster Project - Saguaro National Park (U.S. National Park Service) (nps.gov).

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