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Notes on Reproduction of Crawfish Frogs, *Lithobates areolatus* (Anura: Ranidae) from Oklahoma

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Abstract

I conducted a histological examination of gonadal material from 28 *Lithobates areolatus* collected in Oklahoma. The smallest mature male (sperm in lumina of seminiferous tubules) measured 68 mm SVL. The smallest mature female (in spawning condition) measured 76 mm SVL. As previously reported for *L. areolatus*, reproduction occurs in the early part of the year. One April *L. areolatus* adult female had been removed from the breeding population due to massive ovarian follicular atresia in which mature follicles were invaded by phagocytic follicle cells and yolk granules were replaced by vascular tissue.

The crawfish frog, *Lithobates areolatus* (Baird and Girard, 1852) has a disjunct distribution including Indiana and Iowa, extending southward to the Gulf of Mexico (Powell et al., 2016). The most detailed study on the biology of *L. areolatus* is by Bragg (1953) who reported *L. areolatus* reproduction in Oklahoma occurred in February, March and April. *Lithobates areolatus* are reported to be explosive breeders with a peak of chorusing during the first few days of the breeding season (Dorcas and Gibbons, 2008). There are two currently accepted subspecies of *L. areolatus* in Oklahoma: *L. areolatus circulosus* (northeastern Oklahoma) and *L. areolatus areolatus* (southeastern Oklahoma) (Sievert and Sievert, 2011). Therefore, according to Bragg (1953) my samples from Atoka and McCurtain Counties would be *L. areolatus areolatus*; those from Ottawa County would be *L. areolatus circulosus* and those from Muskogee and Hughes Counties are likely intergrades. Subspecies terminology for *L. areolatus* is compatible with Crother (2017). The two subspecies can interbreed (Sievert and Sievert, 2011). In this paper I present data from a histological examination of *L. areolatus* gonadal material from Oklahoma. Utilization of museum collections for obtaining reproductive data avoids removing additional animals from the wild.

A sample consisting of 28 *L. areolatus* from Oklahoma collected 1944 to 2007 consisting of 20 adult males (mean snout–vent length, SVL = 79.8 mm ± 7.2 SD, range = 68–98 mm), seven adult females (mean SVL = 84 mm ± 5.3 SD, range = 76–91 mm), and one sub-adult female (SVL = 73 mm) was examined from the herpetology collection of the Sam Noble Museum, University of Oklahoma, Norman, Oklahoma, 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 μm and stained with Harris hematoxylin followed by eosin counterstain (Presnell and Schreibman, 1997). Histology slides were deposited at OMNH. An unpaired *t*-test was used to test for differences between male and female SVLs (Instat, vers. 3.0b, Graphpad Software, San Diego, CA).

There was no significant size difference between mean SVL of adult male versus adult females of *L. areolatus* (t = 1.42, df = 25, P = 0.17). The testicular morphology of *L. areolatus* is similar to that of other anurans as described in Ogielska and Bartmañska (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 Bartmañska, 2009a). In *L. areolatus* testes, the heads of the spermatozoans were typically touching the inner boundary of the seminiferous tubule; sperm tails projected into the lumen. In some seminiferous tubules there was a tangled mass of spermatozoa in the lumen. All 20 adult males in my sample exhibited spermiogenesis. By month these were: February (n = 5), March (n = 6), April (n = 8), May (n = 1). The smallest mature male *L. areolatus* measured 68 mm SVL (OMNH 26283) and was from March (Muskogee County). In Wright and Wright (1970) adult males of *L. areolatus* ranged from 63 to 104 mm SVL.

The ovaries of *L. areolatus* 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 Bartmañska, 2009b). Mature oocytes are filled with yolk droplets; the layer of surrounding follicular cells is thinly stretched. The smallest mature *L. areolatus* female (spawning condition) in my OMNH sample measured 76 mm SVL. Adult females of *L. areolatus* ranged from 75 to 113 mm (Wright and Wright, 1970). A slightly smaller female measured 73 mm in SVL (OMNH 42017), contained non-vitellogenic oocytes and was considered to be a subadult.

Although my sample of seven females is too small to describe the ovarian cycle, the presence of ready to spawn females from February to April (Table 1), reconfirms the findings of others (Table 2) of a winter–spring spawning period for *L. areolatus*.

Four of the seven (57%) of the adult *L. areolatus* in my sample contained atretic oocytes. Atresia is a widespread process occurring in the ovaries of all vertebrates (Uribe Aranzábal,

Table 1. Two stages in the ovarian cycle of 7 adult *Lithobates areolatus* females from Oklahoma.

Month	*n*	Ready to spawn	Not in spawning condition
February	1	1	0
March	1	1	0
April	3	2	1
May	1	0	1
June	1	0	1

Table 2. Months of breeding by state for *Lithobates areolatus*.

Locality	Breeding Period	Source
Alabama	February-March	Mount, 1975
Arkansas	Begins in January	Bacon and Anderson, 1976
Arkansas	February to April	Trauth et al., 1990
Illinois	March to April	Phillips et al., 1999
Indiana	March to April	Minton, 2001
Iowa	mid-April	LeClere, 2013
Kansas	March to May	Busby and Brecheisen, 1997
Louisiana	Winter	Boundy and Carr, 2017
Missouri	late February to April	Johnson, 2000
Oklahoma	February to April	Bragg, 1953
Tennessee	February to early May	Niemiller and Reynolds, 2011
Texas	February to June or year-round	Tipton et al., 2012
Not given	February to June	Wright and Wright, 1970

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 follicle cells which invade the follicle and eventually degenerate after accumulating dark pigment (Ogielska and Bartmañska, 2009b). See Saidapur and Nadkarni (1973) and Ogielska et al. (2010) for a detailed description of the stages of follicular atresia in the frog ovary. Follicular atresia is a conspicuous feature of postbreeding ovaries (Saidapur, 1978). Atresia may influence the number of ovulated oocytes (Uribe Aranzábal, 2011) and can remove females from the breeding population (Goldberg, 2017). Two of the mature females, one from April (OMNH 43585, SVL = 88 mm) and one from June (OMNH 42015, SVL = 91 mm) exhibited massive follicular atresia. Mature oocytes in the April female (OMNH 43585) were no longer viable; yolk granules were absent, the interior of the follicle was filled with blood and the inner periphery of the follicle was lined by a layer of enlarged, vacuolated follicle cells. The ovary from June (OMNH 42015) contained numerous small atretic oocytes interspersed with non-vitellogenic oocytes. It is conceivable this *L. areolatus* female may have spawned earlier in the year. However the factors responsible for the massive atresia resulting in destruction of the ovarian follicles in the April female (OMNH 43585), during a time when *L. areolatus* breeds in Oklahoma (Bragg, 1953), are not known.

The causes of follicular atresia in non-mammalian vertebrates are not fully understood although it is associated with captivity, lack of food, crowding and irradiation (Saidapur, 1978). In fish, atresia of follicles may be induced by starvation (Hunter and Macewicz, 1985). Adverse environmental conditions such as starvation and suboptimal lighting may also cause atresia of vitellogenic oocytes in amphibians (Jørgensen, 1992).

Months of and time of reproduction (by state) for *L. areolatus* are shown in Table 2. Breeding is restricted to early in the year throughout its range. The report of year-round reproduction for *L. areolatus* in Texas (Tipton et al., 2012) merits reexamination.

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Literature Cited

Bacon, E. J. Jr., and Z. M. Anderson. 1976. Distributional records of amphibians and reptiles from coastal plain of Arkansas. Journal of the Arkansas Academy of Science 30(7):14-15.

Boundy, J., and J. L. Carr. 2017. Amphibians and reptiles of Louisiana. An identification and reference guide. Baton Rouge: Louisiana State University Press.

Bragg, A. N. 1953. A study of *Rana areolata* in Oklahoma. The Wasmann Journal of Biology 11(3):273-318.

Busby, W. H., and W. R. Brecheisen. 1997. Chorusing phenology and habitat associations of the crawfish frog, *Rana areolata* (Anura: Ranidae), in Kansas. The Southwestern Naturalist 42(2):210-217.

Crother, B. I. (editor). 2017. Scientific and standard English names of amphibians and reptiles of North America north of Mexico, with comments regarding confidence in our understanding. Eighth edition. Society for the Study of Amphibians and Reptiles Herpetological Circular 43:1-102.

Dorcas, M. E., and W. Gibbons. 2008. Frogs and toads of the southeast. Athens: The University of Georgia Press.

Goldberg, S. R. 2017. Notes on reproduction of California treefrogs, *Hyliola cadaverina* (Anura: Hylidae) from Riverside County, California. Sonoran Herpetologist 30(1):5-7.

Hunter, J. R., and B. J. Macewicz. 1985. Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax*. Fishery Bulletin 83(2):119-136.

Johnson, T. R. 2000. The amphibians and reptiles of Missouri. Second edition. Jefferson City, Missouri: Missouri Department of Conservation.

Jørgensen, C. B. 1992. Growth and reproduction. Pp. 439-466. *In*: M. E. Feder and W. W. Burggren, editors, Environmental physiology of the amphibians. Chicago: The University of Chicago Press.

LeClere, J. B. 2013. A field guide to the amphibians and reptiles of Iowa. Rodeo, New Mexico: Eco Herpetological Publishing and Distribution.

Minton, S. A., Jr. 2001. Amphibians and reptiles of Indiana. Second edition. Indianapolis: Indiana Academy of Science.

Mount, R. H. 1975. The reptiles and amphibians of Alabama. Tuscaloosa: University of Alabama Press.

Niemiller, M. L., and R. G. Reynolds. 2011. The amphibians of Tennessee. Knoxville: University of Tennessee Press.

Ogielska, M., and J. Bartmañska. 2009a. Spermatogenesis and male reproductive system in Amphibia—Anura. Pp. 34-99. *In*: M. Ogielska, editor, Reproduction of amphibians. Enfield, New Hampshire: Science Publishers.

Ogielska, M., and J. Bartmañska. 2009b. Oogenesis and female reproductive system in Amphibia—Anura. Pp. 153-272. *In*: M. Ogielska, editor, Reproduction of amphibians. Enfield, New Hampshire: Science Publishers.

Ogielska, M., B. Rozenblut, R. Augustyñska and A. Kotusz. 2010. Degeneration of germ line cells in amphibian ovary. Acta Zoologica (Stockholm) 91(3):319-327.

Phillips, C. A., R. A. Brandon and E. O. Moll. 1999. Field guide to amphibians and reptiles of Illinois. Champaign: Illinois Natural History Survey Manual 8.

Powell, R., R. Conant, and J. T. Collins. 2016. Peterson field guide to reptiles and amphibians of eastern and central North America. Fourth edition. Boston: Houghton, Mifflin, Harcourt.

Presnell, J. K., and M. P. Schreibman. 1997. Humason's animal tissue techniques. Fifth edition. Baltimore: The Johns Hopkins University Press.

Saidapur, S. K. 1978. Follicular atresia in the ovaries of nonmamnalian vertebrates. Pp. 225-244. *In*: G. H. Bourne, J. F. Danielli and K. W. Jeon, editors, International Review of Cytology, Vol. 54. New York: Academic Press.

Saidapur, S. K., and V. B. Nadkarni. 1973. Follicular atresia in the ovary of the frog *Rana cyanophlyctis* (Schneider). Acta Anatomica 86(3-4):559-564.

Sievert G., and L. Sievert. 2011. A field guide to Oklahoma's amphibians and reptiles. Oklahoma City: Oklahoma Department of Wildlife Conservation.

Tipton, B. L., T. L. Hibbitts, T. D. Hibbitts, T. J. Hibbitts and T. J. LaDuc. 2012. Texas amphibians: A field guide. Austin: University of Texas Press.

Trauth, S. E., R. L. Cox, Jr., B. P. Butterfield, D. A. Sauhey and W. E. Meshaka, Jr. 1990. Reproductive phenophases and clutch characteristics of selected Arkansas amphibians. Journal of the Arkansas Academy of Science 44(29):107-113.

Uribe Aranzábal, M. C. 2009. Oogenesis and female reproductive system in Amphibia—Urodela. Pp. 273-304. *In*: M. Ogielska, editor, Reproduction of amphibians. Enfield, New Hampshire: Science Publishers.

————. 2011. Hormones and the female reproductive system of amphibians. Pp. 55-81 *In*: D. O. Norris and K. H. Lopez, editors, Hormones and reproduction of vertebrates, Volume 2. Amphibians. Amsterdam: Elsevier.

Wright, A. H., and A. A. Wright. 1970. Handbook of frogs and toads of the United States and Canada. Ithaca, New York: Comstock Publishing Associates.

Appendix

Twenty-eight *Lithobates areolatus* from Oklahoma examined (by county) from the herpetology collection of the Sam Noble Museum (OMNH), The University of Oklahoma, Norman, Oklahoma, USA.

Atoka: OMNH 39111, 39112, 39470, 39471, 39826, 42015–42017, 42021; **Bryan:** OMNH 43585; **Delaware:** OMNH 26313, 48041, 48042; **Hughes:** OMNH 38156; **McCurtain:** OMNH 26270, 43863, 43864, 43866–43868, 43871, 43873; **Muskogee:** OMNH 26283; **Ottawa:** OMNH 27605, 27606; **Seminole:** OMNH 43713; **Tulsa:** OMNH 30916; **Washington:** OMNH 39878.