Age Estimation using Phalangeal Skeletochronology in Northern Crawfish Frogs, *Lithobates areolatus circulosus* (Amphibia: Anura: Ranidae), from Arkansas

S.E. Trauth[1] and C.S. Thigpen[2]

[1]*Department of Biological Sciences, Arkansas State University (Emeritus), P.O. Box 599, State University, AR 72467*
[2]*Department of Biological Sciences, Arkansas State University, P.O. Box 599, State University, AR 72467*

*Correspondence: strauth@astate.edu

Running Title: Phalangeal Skeletochronology in the Northern Crawfish Frog

As an obligate crayfish burrow dweller, crawfish frogs have historically occupied a relatively narrow ecological niche throughout their distribution in the tall grass prairies and grasslands of the central and south-central United States (Redmer 2000; Powell *et al.* 2016; Lannoo *et al.* 2018). Habitat loss and shifting climate patterns pose as major threats to the continued existence of this species (Lannoo and Stiles 2017). In Arkansas, the Northern Crawfish Frog, *Lithobates areolatus circulosus*, occurs in only 19 of its 75 counties (Trauth *et al.* 2004; Trauth and Holt 2017). Because of their secretive nature, late winter-early spring breeding season, and current protected status by the Arkansas Game and Fish Commission, this species remains a rarity in most museum collections in the state (Trauth *et al.* 2004). Moreover, only anecdotal information exits regarding any aspect of their natural history in Arkansas (Trauth *et al.* 1990).

In the present study, we chose to conduct a phalangeal skeletochronological investigation of the Northern Crawfish Frog utilizing museum specimens (n = 10) deposited in the herpetological collection (ASUMZ 13900, 14150, 31084-86, 33746-49, 33611) housed in the Arkansas Center for Biodiversity Collections located at Arkansas State University. Four frogs included in this sample were recently collected by SET while road cruising on Turkey Pond Loop (35.2147195N, 92.7567921W) in Conway County on the 20th and 27th February, 2018. Our goals were to estimate the age of individuals within this small Arkansas frog sample by counting annular lines of arrested growth (LAGs) and compare these results with the age estimates found for this frog by Redmer (2000), who utilized the same histological technique on a crawfish frog population in southern Illinois.

The distal phalanx (Fig. 1) of the 4th toe of the left hind foot from each frog was removed and placed into either 70% ethanol (historic specimens) or 10% neutral buffered formalin for fresh toes. Then, we treated all toes with a decalcifying solution (1% hydrochloric acid)

Figure 1. Photomicrograph of a transverse section through the distal phalanx of *Lithobates areolatus circulosus* (ASUMZ 33748) revealing phalangeal bone (Pb). Sm = striated muscle.

for 48 h. Next, we prepared the phalanxes for examination by light microscopy using standard histological methods (Presnell and Schreibman 1997). We dehydrated the toes in a graded series of ethanol solutions (70-100%), cleared each toe with xylene (50-100%), and infiltrated the toe segment in paraffin in a 56 °C convection oven overnight. We then embedded each toe into a paraffin mold for hardening, which was followed by block trimming and mounting for sectioning using a American Optical rotary microtome. We histosectioned each toe phalanx transversely into 10 µm serial ribbons and adhered these ribbons to microscope slides with Haupt's adhesive. We transferred the slides through staining dishes containing hematoxylin and eosin (H&E). One of us (SET) photographed the toes using a Leica DM 2000 LED light

microscope. All slides are currently in the possession of SET.

The toes of 8 males and 2 females were examined and yielded the following results (ASUMZ no., collection date, snout-vent length—SVL, gender, and estimated age in yrs.): 1) ASUMZ 13900, 7 March 1987, 71, male, 1; 2) ASUMZ 14150, 14 March 1989, 80, male, 2; 3) ASUMZ 31085, 29 April 2008, 96, male, 3; 4) ASUMZ 31086, 29 April 2008, 92, male, 3; 5) ASUMZ 33746, 20 February 2018, 75, male, 3; 6) ASUMZ 33748, 27 March 2018, 88, male, 4; 7) ASUMZ 33749, 20 February 2018, 88, 4; 8) ASUMZ 33747, 20 February 2018, 105, female, 5; 9) ASUMZ 31084, 1 January 1966, 111, female, 4, and 10) ASUMZ 33611, 28 February 2017, 120, male, 8.

Our skeletochronological results were generally similar to the age-body length distributions reported by Redmer (2000) for his crawfish frog population from southern Illinois. Except for our largest male (10), older males (6, 7; Fig. 1A, B) exhibited 4 LAGs, and this estimated age matched well with most of the body sizes of 4-year-old males found in Illinois. Two of our 3-year-old males (3, 4) had slightly larger body sizes compared to the Illinois sample (71 – 86 mm SVL). These larger body sizes possibly indicate growth following reproductive activity. Redmer (2000) had no 1-year-old males in his sample. Our 1-year-old male matched in size to his 2-year-old males. Our oldest female (8) was 5 years old (Fig. 1C). Except for our oldest male (Fig. 3), all body sizes were comparable to values found for frogs reported by Redmer (2000). In addition, 8 years, as observed in our oldest male, was near the maximum lifespan of 9 years documented for this species (Mike Lannoo *pers. comm*.).

Skeletochronology can show increments of annual growth as well as cyclic growth patterns in bones. These patterns often reflect seasonal changes in feeding activity resulting from dramatic shifts in climatic events. For example, the male in Fig. 2B exhibited a large growth increment in year 1, which was then followed by a much smaller increment in year 2. The growth rings seen in years 3 and 4 are, in turn, even smaller than year 2 but similar to one another. In contrast, the variations in the growth pattern of the female shown in Fig. 2C are striking with substantial growth in year 2 followed by greatly reduced growth in years 3 and 4. Then, growth in year 5 showed a two-fold increase compared to the two previous years.

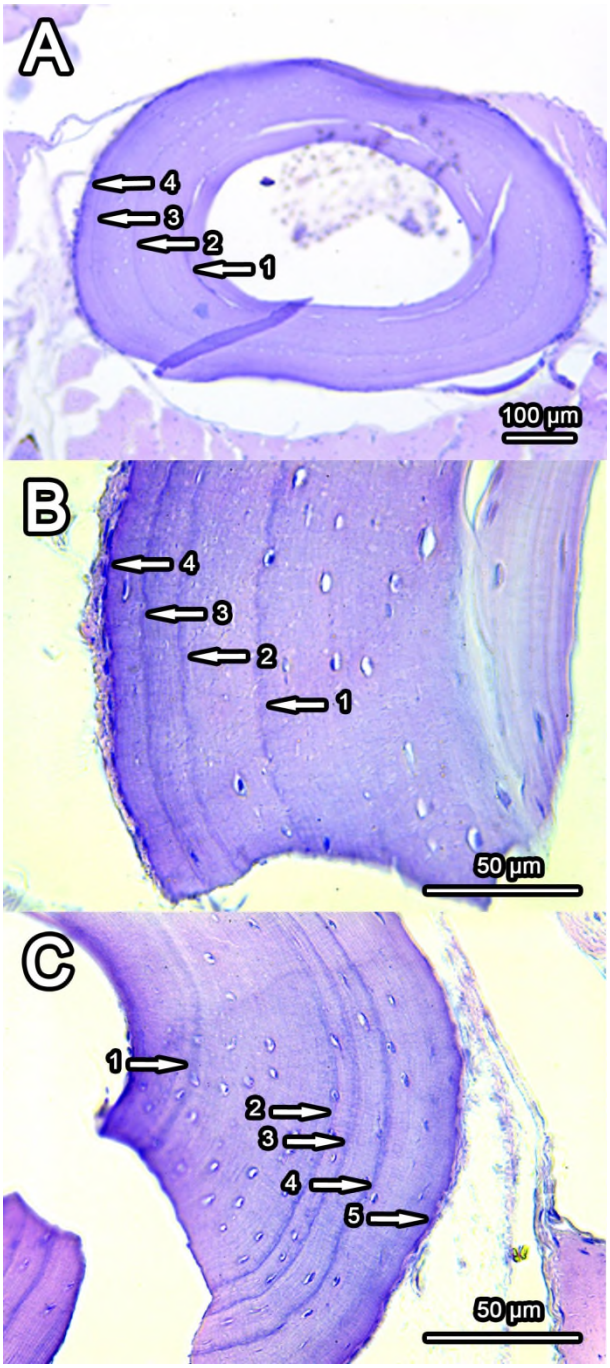

Figure 2. Photomicrographs of transverse sections through the distal phalanxes of 3 *Lithobates areolatus circulosus* revealing lines of arrested growth (ends of arrows). A. ASUMZ 33748. B. ASUMZ 33749. C. ASUMZ 33747.

Acknowledgments

We thank the Arkansas Game and Fish Commission for issuing the scientific collection permits to SET (nos. 012020161 and 020520182).

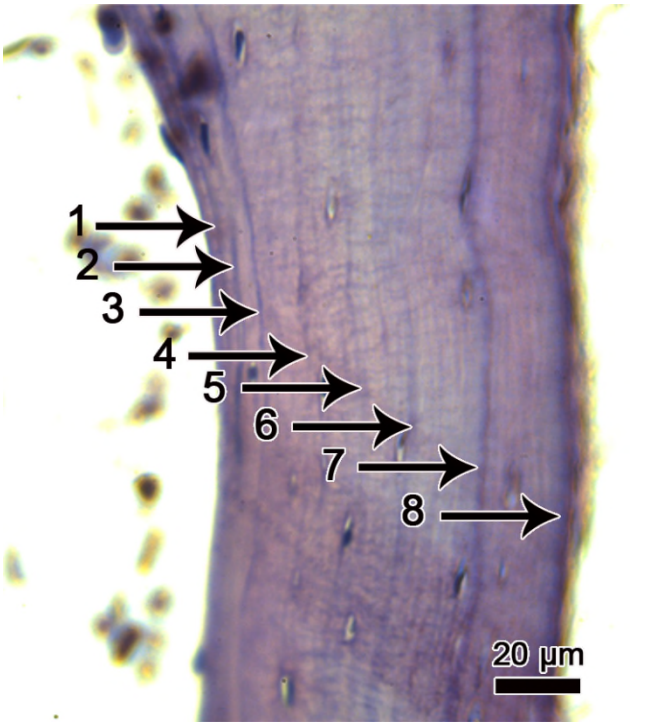

Figure 3. Photomicrograph of a transverse section through a distal phalanx of *Lithobates areolatus circulosus* revealing 8 lines of arrested growth (ends of arrows) in ASUMZ 33611.

Literature Cited

Lannoo MJ and **RM Stiles.** 2017. Effects of short-term climate variation on a long-lived frog. Copeia 105:726-33.

Lannoo MJ, RM Stiles, D Saenz, and **TJ Hibbitts.** 2018. Comparative call characteristics in the anuran subgenus *Nenirana.* Copeia 106:575-79.

Powell R, R Conant, and **JT Collins.** 2016. Peterson field guide to reptiles and amphibians of eastern and central North America. 4th ed. Houghton Mifflin Harcourt Co. (NY). 494 p.

Presnell JK and **MP Schreibman.** 1997. Humason's Animal Tissue Techniques. 5th ed. The Johns Hopkins University Press. (Baltimore MD). 572 p.

Redmer R. 2000. Demographic and reproductive characteristics of a southern Illinois population of the crayfish frog, *Rana areolata.* Journal of the Iowa Academy of Science 107:128-133.

Trauth SE and **A Holt**. 2017. *Lithobates areolatus circulosus* (Northern Crawfish Frog). Geographic distribution. Herpetological Review 58:383.

Trauth SE, HW Robison, and **MV Plummer.** 2004. The amphibians and reptiles of Arkansas. University of Arkansas Press (Fayetteville, AR). 421 p.

Trauth SE, RL Cox, Jr., BP Butterfield, DA Saugey, and **WE Meshaka.** 1990. Reproductive phenophases and clutch characteristics of selected Arkansas amphibians. Proceedings of the Arkansas Academy of Science 48:196-209.

.