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Size and Age Records for an Arkansas Specimen of the American Bullfrog, Lithobates catesbeianus (Anura: Ranidae)

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Running Title: Size and Age Records for an Arkansas Specimen of Lithobates catesbeianus (Anura: Ranidae)

The American Bullfrog (Lithobates catesbeianus), North America's largest anuran, is widely distributed throughout the lower 48 United States, and populations have been introduced into western Canada and Hawaii (Dodd 2013) as well as many other regions of the world (Kraus 2009), such as China (Wang and Li 2009). This species occurs in nearly every county in Arkansas (Trauth et al. 2004), and is a prized game animal. The largest body size ever recorded for the American Bullfrog is 204.2 mm snout-vent length (SVL), measured from an adult female collected from Cleveland County, Oklahoma in 1995; its mass was 908.6 g (Lutterschmidt et al. 1996). In Arkansas, the body size normally ranges from 90-152 mm SVL (McKamie and Heidt 1974, Trauth et al. 2004) but, in rare instances, can reach nearly 190 mm. In the following, we report on the largest known specimen of this species ever reported from Arkansas and provide an estimate of its age using skeletochronology.

Skeletochronology, a histological technique for determining annual growth increments by counting lines of arrested growth (LAGs) in bones in temperate amphibians and reptiles, has been shown to be a reliable method for age determination (Castanet and number Smirina 1990). А of recent skeletochronological studies have used ranid frogs Bastien and Leclair 1992, Tsiora and (e.g., Kyriakopoulou-Sklavounou 2002, Lai et al. 2005, Kyriakopoulou-Sklavounou et al. 2008, Liao 2011, Sarasola-Puente et al. 2011) to determine growth, size, age of maturity, and longevity in frog populations throughout the world.

On April 27, 2013, one of us (TAW) collected a gravid female American Bullfrog (Fig. 1) from Gum Slough Ditch at its intersection with St. Hwy 230 (35°, 54', 33.53"N; 90°, 50', 50.78"W) approximately 3.6 km west of Bono (Craighead County), Arkansas. The specimen was brought to the Department of Biological Sciences at Arkansas State University, photographed, and massed (784.5 g). Its body length measured 195

mm from snout tip to groin. The frog was deposited into the Arkansas State University herpetological collection (ASUMZ 32687).

In order to determine an age estimate of this specimen, the diaphyseal portion of the left tibiofibula and a phalangeal segment of the 2^{nd} digit of the right pes were removed and placed into decalcifying agent (1% v/v hydrochloric acid) for 3 days and then



Figure 1. Adult female American Bullfrog, *Lithobates catesbeianus* (ASUMZ 32687), from Craighead County, Arkansas. A. Left lateral view of specimen. B. Ventral surface of specimen lying supine on a metric ruler; arrow points to 220 mm.

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transferred into 50% v/v ethanol for temporary storage. Later, these bones were dehydrated in a series of graded ethanol solutions in preparation for paraffin infiltration and embedding. Bones were then sectioned with a rotary microtome at a thickness of 10 μ m and affixed to microscope slides using Haupt's adhesive. Slides were stained with hematoxylin (6 min) and eosin (45 sec) using a standard histological protocol (Presnell and Schreibman 1997). A Nikon Eclipse 600 epi-fluorescent light microscope with a Nikon DXM 1200C digital camera was utilized to obtain photomicrographs.



Figure 2. Transverse sections of different regions of the diaphysis of the tibiofibula of the adult female American Bullfrog, *Lithobates catesbeianus* (ASUMZ 32687). A. Arrows point to lines of arrested growth (LAGs); scale bar = $50 \mu m$. B. Similar sectional plane as shown in A. Lines link LAGs between A and B. ML = metamorphosis line; P = periosteum.

The results of skeletochronology of the tibiofibula of ASUMZ 32687 revealed an age estimate of six yr based upon number of LAGS (Fig. 2). In addition, no endosteal resorption was evident. Several false LAGs were observed, however, and these were especially evident during the first year of life and also during year 2 through 4 (see Fig. 2B). The maximum age of an American Bullfrog is 7 years, 3 months and 24 days, recorded from a specimen held in captivity at the Philadelphia Zoo (Snider and Bowler 1992).

Acknowledgments

Deposition of the American Bullfrog into the Arkansas State University herpetological collection was authorized by the Arkansas Game and Fish Commission under Scientific Collection Permit No. 020520134 to SET.

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