

Absence of Lines of Arrested Growth in Overwintered Tadpoles of the American Bullfrog, *Rana catesbeiana* (Amphibia, Anura)

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Abstract: In order to ascertain formation of lines of arrested growth (LAGs) in anuran larvae, cross-sections of long bones in overwintered tadpoles and juveniles of *Rana catesbeiana* were observed. However, no LAGs were evident in these individuals, although we could observe clear LAGs in adults treated in the same way. The result contrasts to those reported for urodelan larvae. We discuss the causes of this phenomenon on the assumption that the absence of LAGs in tadpoles is common to other anurans as well.

Key words: Overwintered tadpoles; *Rana catesbeiana*; LAG formation; Skeleto-chronology

INTRODUCTION

In a study of *Hynobius kimurae*, Misawa and Matsui (1999) found lines of arrested growth (LAGs) formed in limb bones of overwintered larvae. However, no studies have reported the occurrence of LAGs in overwintered anuran tadpoles, although formation of LAGs in bones of many postmetamorphic anuran species has been well documented (e.g., Castanet and Smirina, 1990; Esteban et al., 1996).

Rana catesbeiana normally hibernates in larval form for at least one winter both in its original habitat (eastern USA: e.g., Viparina and Just, 1975) and in introduced regions (e.g., Japan: Maeda and Matsui, 1999). Meta-

morphs of this species also often hibernate in the water, under mud at the bottom of ponds (Maeda and Matsui, 1999). Thus, this species offers a good opportunity to examine the condition of LAGs in the larval stage as compared to that after metamorphosis in anurans.

MATERIALS AND METHODS

Forty-three overwintered tadpoles (stage 39–46 of Gosner, 1960) and three metamorphs were collected between 25 and 30 July 1998 from a permanent pond called Bungaike, Kizu-cho, Kyoto Prefecture. For comparisons, we also collected a metamorphosed juvenile on 10 September 1998 and a gravid female on 23 June 1998 from around the same pond. Whole bodies in the case of tadpoles and small juveniles or digital bones in larger individuals were first fixed in 10% formalin, and preserved in 70% ethanol until study. Fifty-nine toe and

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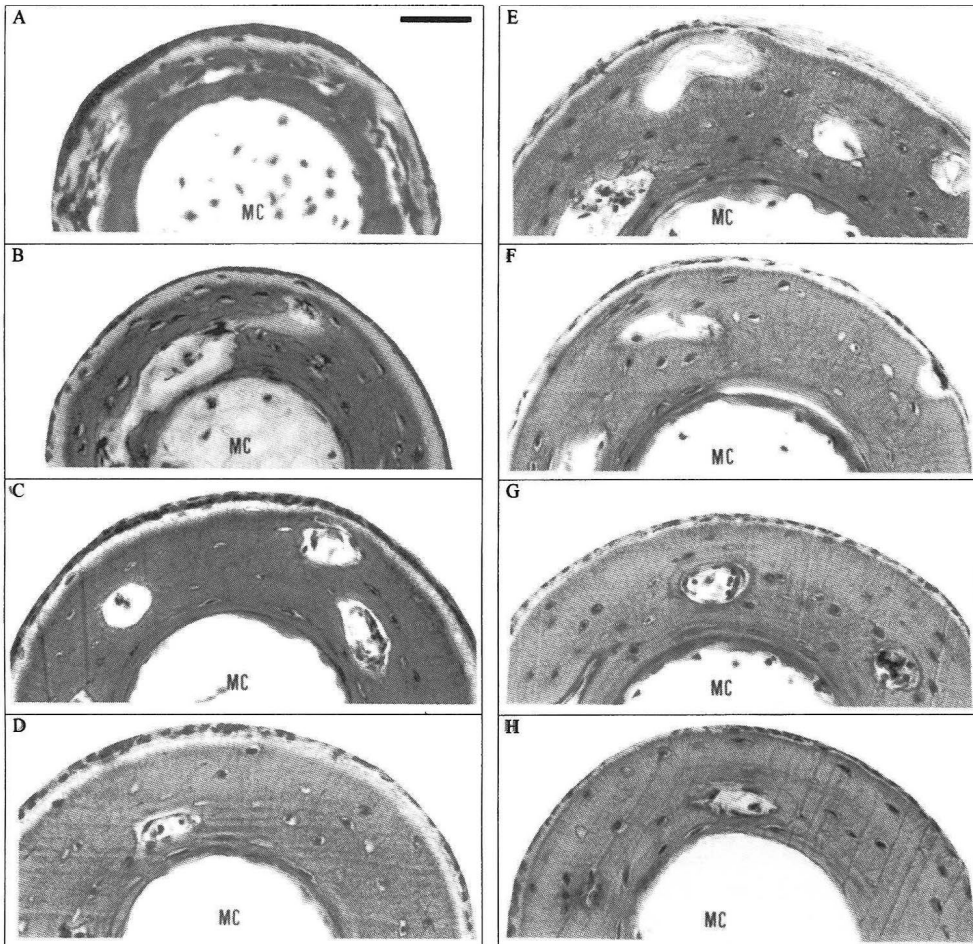


FIG. 1. Phalangeal cross section of overwintered *Rana catesbeiana* tadpoles; (A) stage 39 (Gosner, 1960) with 39.8 mm body length (BL) collected on 25 July 1998, (B) stage 40 with 43.7 mm BL collected on 25 July 1998, (C) stage 41 with 38.4 mm BL collected on 25 July 1998, (D) stage 42 with 42.3 mm BL collected on 25 July 1998, (E) stage 43 with 41.4 mm BL collected on 28 July 1998, (F) stage 44 with 39.7 mm BL collected on 30 July 1998, (G) stage 45 with 41.0 mm BL collected on 28 July 1998, (H) stage 46 with 43.2 mm BL collected on 30 July 1998. All sections show no lines of arrested growth in bones. MC=marrow cavity. Scale bar=50 μ m.

femoral bones and three finger bones were removed from the tadpoles and metamorphs, respectively, and were decalcified in 5% nitric acid for 120 min. They were washed in running water for one night before and after this process. Then the bones were sectioned on a

freezing microtome and stained with hematoxylin (Mayer's acid hemalum) for 30 min. We selected sections which included the diaphysis region, and mounted them in glycerin for microscopic examination.

RESULTS

The overwintered tadpoles varied in average body length (mean \pm 2SE; in mm) from 39.9 (N=1) at stage 39 to 43.3 \pm 0.96 (N=11) at stage 46 (Table 1). Completely metamorphosed froglets varied in snout-vent length (SVL) from 46.3 to 57.3 mm (N=3). Cross sections of digital bones in all individuals of overwintered tadpoles (Figs. 1A–H) and completely metamorphosed froglets exhibited incomplete ossification with many holes. There were no LAGs in any of these sections. Although a metamorphosed line (ML: Hemelaar, 1985) was found in an individual that was collected shortly after emergence from hibernation, we did not find any lines in any other individuals. A juvenile of 86.1 mm SVL collected in early September also lacked LAGs (Fig. 2A) and showed a very rapid remodeling of the finger bone. Minimum

diameter of the resorption line (RL, irregular, inner surface of the finger bone: Hemelaar and van Gelder, 1980) in this juvenile was always

TABLE 1. Developmental stage (Gosner, 1960), number of individuals (N), and body length (mean \pm 2SE in mm) of overwintered *Rana catesbeiana* tadpoles.

Stage	N	Body length
39	1	39.8
40	5	41.3 \pm 2.89
41	2	40.6
42	5	41.9 \pm 3.95
43	6	41.0 \pm 1.77
44	6	41.7 \pm 1.84
45	7	42.1 \pm 1.28
46	11	43.3 \pm 0.96

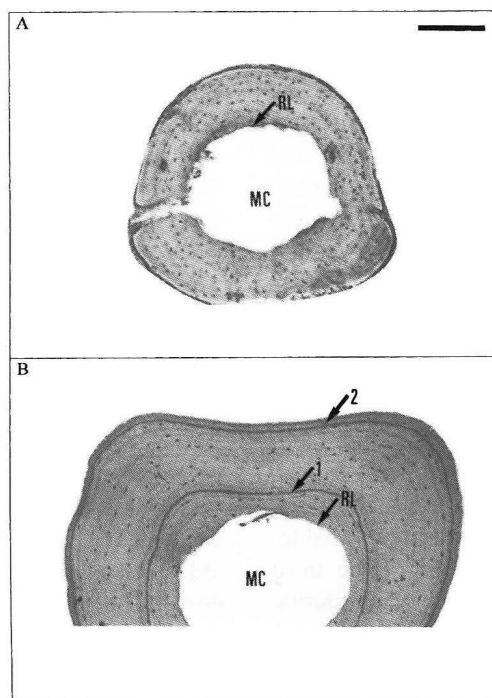


FIG. 2. Phalangeal cross section of *Rana catesbeiana*; (A) juvenile with 86.1 mm SVL collected on 10 September 1998 showing no lines of arrested growth (LAGs), but the resorption line (RL), (B) adult female with 143.8 mm SVL collected on 23 June 1998 showing two LAGs besides RL. MC=marrow cavity. Scale bar=200 μ m.

larger than outer diameter of fingers in froglets which had just completed metamorphosis. On the other hand, two clear LAGs were observed in the adult frog (Fig. 2B).

DISCUSSION

From the time of capture and body size, it is obvious that the tadpoles and froglets we examined had gone through at least one overwintering. In some anuran species hitherto studied (e.g., *Rana tagoi* and *R. sakurarii*: Kusano et al., 1995a, b; *R. nigromaculata*: Khonsue et al., unpublished data), formation of LAGs occurs fairly late after the emergence of frogs from hibernation. However, the dates of collection of our samples (25–30 July and 10 September 1998) seem to be late enough for the formation of a new ring, and the larvae and juveniles examined must have had a chance to increase bony material after overwintering, because, unlike adults, they did not need to expend energy for special activities such as reproduction.

Previous skeletochronological studies on amphibians usually resulted in detection of clear LAGs in bones of both juveniles and adults (e.g. Hemelaar, 1985; see review in Castanet and Smirina, 1990). Similarly, formation of LAGs has been confirmed in overwintered larvae in at least one species of urodela (Mizawa and Matsui, 1999). Thus, the absence of LAGs in overwintered tadpoles of *R. catesbeiana* is surprising. This discovery, though whether specific to *R. catesbeiana* or common to other anuran species is not yet determined, suggests a different process of LAG formation between the orders Urodela and Anura. Although only data for adults are available, *Typhlonectes natans*, a member of the third amphibian order, Gymnophiona, also exhibits LAGs (Measey and Wilkinson, 1998). Species of this genus spend a totally aquatic life even after metamorphosis (Taylor, 1968), and prove that life in the water by itself does not prevent the formation of LAGs. Why are there no LAGs in bones of overwintered *R. catesbeiana* tadpoles or juveniles, unlike in urodeles?

One possible reason may be related to the physiological differences between these two orders. In urodelan larvae, as is clear from the rapid development of forelimbs long before metamorphosis, limbs are more important for locomotion than in anuran larvae. Hence calcium deposition, which is essential in the formation of LAGs in limb bones, would occur much earlier in urodelan larvae than in anuran larvae.

In *Rana saharica* from a desert of Morocco, Esteban et al. (1999) found that some froglets lacked LAGs, unlike adults. They considered that the froglets may have hibernated before the start of the ossification process which was vital to LAG formation.

The pond where our tadpoles were collected is filled with deep water (deepest point >3 m) all year round, and the temperatures at the bottom of this pond, where the tadpoles must have stayed throughout hibernation, might be more constant than in the surface stratum or on the surrounding banks. Such a stability of the ambient temperature might have a role in blocking the formation of LAGs.

However, metamorphs of *R. catesbeiana* are also reported to usually hibernate in the water (Maeda and Matsui, 1999), and we assume this might have been the case with our samples. They still showed the formation of LAGs. Therefore, as suggested by Esteban et al. (1999), the absence of LAGs in overwintered tadpoles and juveniles of *R. catesbeiana* might be affected by age-dependent timing of the strat of hibernation and ossification. We need further ecological, ethological, and histological studies to solve this problem.

Finally, what has become clear from this study is that the number of LAGs found in older juveniles and adults of *R. catesbeiana* does not represent their actual age from the time of egg laying but their age after metamorphosis.

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LITERATURE CITED

- CASTANET, J. AND E. SMIRINA. 1990. Introduction to the skeletochronological method in amphibians and reptiles. *Annal. Sci. Nat. Zool.* 11: 191–196.
- ESTEBAN, M., M. GARCIA-PARIS, AND J. CASTANET. 1996. Use of bone histology in estimating the age of frogs (*Rana perezi*) from a warm temperate climate area. *Can. J. Zool.* 74: 1914–1921.
- ESTEBAN, M., M. GARCIA-PARIS, D. BUCKLEY, AND J. CASTANET. 1999. Bone growth and age in *Rana saharica*, a water frog living in a desert environment. *Annal. Zool. Frennici* 36: 53–62.
- GOSNER, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183–190.
- HEMELAAR, A. 1985. An improved method to estimate the number of year rings resorbed in phalanges of *Bufo bufo* (L.) and its application to populations from different latitudes and altitudes. *Amphibia-Reptilia* 6(4): 323–341.
- HEMELAAR, A. S. M. AND J. J. VAN GELDER. 1980. Annual growth rings in phalanges of *Bufo bufo* (Anura, Amphibia) from the Netherlands and their use for age determination. *Neth. J. Zool.* 30: 129–135.
- KUSANO, T., K. FUKUYAMA, AND N. MIYASHITA. 1995a. Age determination of the stream frog, *Rana sakuraii*, by skeletochronology. *J. Herpetol.* 29(4): 625–628.
- KUSANO, T., K. FUKUYAMA, AND N. MIYASHITA. 1995b. Body size and age determination by skeletochronology of the brown frog, *Rana tagoi*, in southwestern Kanto. *Jpn. J. Herpetol.* 16(2): 29–34.
- MAEDA, N. AND M. MATSUI. 1999. Frogs and Toads of Japan, Rev. Ed. Bun-ichi Sogo Shuppan, Tokyo. pp. 100–107.
- MEASEY, G. J. AND M. WILKINSON. 1998. Lines of arrested growth in the caecilian, *Typhlonectes natans* (Amphibia: Gymnophiona). *Amphibia-Reptilia* 19(1): 91–95.
- MISAWA, Y. AND M. MATSUI. 1999. Age determination by skeletochronology of the Japanese salamander *Hynobius kimurae* (Amphibia, Urodela). *Zool. Sci.* 16(5): 845–851.
- TAYLOR, E. H. 1968. *The Caecilians of the World*. Univ. Kansas Press, Lawrence, Kansas. v+viii+848 p.
- VIPARINA, S. AND J. J. JUST. 1975. The life period, growth and differentiation of *Rana catesbeiana* larvae occurring in nature. *Copeia* 1975(1): 103–109.