



Age and growth examined by skeletochronology for the stream-dwelling frog *Feirana taihangnicus* in central China

Lixia Zhang¹, Youqiang Lu^{1,2}, Xin Lu³ & Xiaohong Chen¹

¹Department of Zoology, College of Life Sciences, Henan Normal University, Xinxiang 453007, China, ²Huarui College of Xinyang Normal University, Xinyang 464000, China, ³Department of Zoology, College of Life Sciences, Wuhan University, Wuhan 430072, China

Feirana taihangnicus is a stream-living frog endemic to central China. We determined the demography of a population from north Henan province using skeletochronology. Males were smaller than females (67.1±1.0 and 77.8±1.0 mm snout vent length, respectively), and began reproduction earlier after metamorphosis (2 and 3 years of age, respectively). Accordingly, males attained younger ages (mean age 5.5±0.3 compared to 6.3±0.2 years in females), reached a shorter maximum lifespan (9 years compared to 10 years in females) and grew at a faster rate (von Bertalanffy's growth coefficient: 0.25 compared to 0.16 in females). Because tadpoles take 2–3 years to complete metamorphosis, these data suggest that *F. taihangnicus* spends at least 5–6 years between the egg stage and reaching adult. The slow life history and resultant low population turnover rates highlight a conservation concern for this high-elevation species.

Key words: body size, *Feirana taihangnicus*, life history, protection, skeletochronological age

INTRODUCTION

Knowledge of the age and growth of individuals belonging to a population critically contributes to our understanding of population dynamics and life history evolution, and is also useful for species conservation. For amphibians, data on age and growth may be obtained through capture-mark-recapture. However, this method is often labour-intensive and affected by high mortality rates of juveniles. Alternatively, skeletochronology may determine the actual age of free-living amphibians through counting annual growth rings in bone tissue, and thus can overcome the major disadvantages of capture-mark-recapture (Halliday & Verrell, 1988). Although skeletochronology is a widely accepted standard technique, it has only been applied for a small fraction of amphibian species (5.3% of 624 currently recognized species of urodeles, Zhang & Lu, 2013a; 1.4% of 6167 species of anurans, Zhang & Lu, 2012).

Feirana taihangnicus is endemic to central China (Fei et al., 2009; Yang et al., 2011), and has a range of only 30,000 km². As a dweller in mountainous streams at elevations from 400 to 1600 m with communal breeding behaviour, strong fidelity to oviposition sites and prolonged larval period, this species is particularly sensitive to habitat alteration (Chen et al., 2011; Zhang et al., 2012; see Hero et al., 2005; Cooper et al., 2008 for high-elevation frogs in general). Here we use skeletochronology to report on age and growth of *F. taihangnicus*, aiming to better understand the life history of this stream dweller and to aid its future conservation.

MATERIALS AND METHODS

This study was conducted in Heilonggou in the Taihangshan National Nature Reserve (35° 16'N, 112° 04'E, 762–948 m elevation), north Henan province, China. The annual average air temperature at an elevation of 760 m is approximately 11°C, with total annual precipitation of about 700 mm and temperature as well as rainfall peaks in summer (June–August). *Feirana taihangnicus* is confined to perennial streams surrounded by temperate coniferous and broad-leaved mixed forests. Spawning activity lasts about one week between late April and early May. Oviposition sites are located in sun-exposed stream sections, where water flows slowly (Chen et al., 2011). Unlike most anurans, amplexus does not occur in this species; instead, up to 15 males (8.7 on average) attempt to fertilize egg clutches deposited by a single female beneath rocks (Chen et al., 2011). Simultaneous polyandry with multiple males not engaged in amplexus is uncommon among anuran species (Roberts & Byrne, 2011). The average clutch size (425 eggs, range: 302–611) is positively correlated with female body size, and the diameter of eggs is on average 3.63 mm (Chen et al., 2011).

During the breeding seasons of 2005, 2006 and 2010, we walked along stream banks and caught frogs by hand during the night. Frogs were sexed and aged (juvenile or adult) immediately after capture. Breeding males of *F. taihangnicus* have no nuptial pads but can be identified through white dorsal tubercles, whereas breeding females are characterized by an inflated abdomen containing eggs. Body size (snout vent length, SVL) was

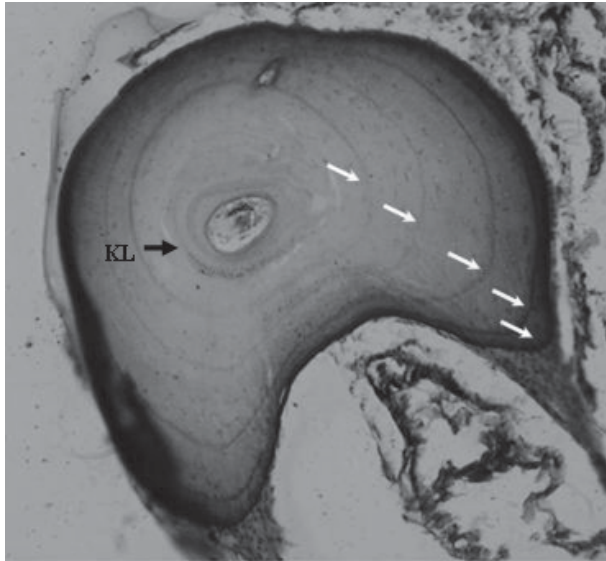


Fig. 1. The histological section of the longest phalanges of a 5-year-old *Feirana taihangnicus* specimen. White arrows indicate the lines of arrested growth; Black arrow indicates the Kastschenko line (KL).

measured with digital calipers to the nearest 0.1 mm. The longest phalanges of the left hindlimb were removed and preserved in 10% formalin for skeletochronology. All frogs examined during the breeding season (27 males, 55 females and 2 juveniles) were released *in situ*. We also included 41 museum specimens collected in this area between May and October 2002–2010 in our analysis. Since secondary sexual traits are absent after the breeding period, these specimens were dissected to identify their sex (16 males, 25 females and 3 juveniles). Such a female-biased sex ratio has been reported previously (Chen et al., 2011).

Histological sections followed the laboratory protocols established by Lu et al. (2006). All specimens showed clear lines of arrested growth (LAGs, Fig. 1). Potential errors in determining age via LAGs might come from resorption of initial LAGs, as well as false lines. However, resorption likely did not occur in this study population, as evidenced by a Kastschenko Line (KL, the division line between the endosteal and periosteal zones; Rozenblut & Ogielska, 2005) present in all individuals examined. False lines were observed in one male and four females, but they were

unclear or incomplete and distinguishable from LAGs. Wagner et al. (2011) considered that skeletochronology may underestimate the true age of amphibians with long lifespans, typically for salamanders where the individual maximum age of more than 10 years is common (ca. 50% of known-age species, Zhang & Lu, 2013a). This should not pose a serious problem for *F. taihangnicus*, due to maximum age being below 10 years (ca. 15%, Zhang & Lu, 2012). Unlike most anuran species (Ma et al., 2009; Chen & Lu, 2011; Liao & Lu, 2011), the profile of cross-sections of phalanges in *F. taihangnicus* was concave due to an embedded tendon, a putative morphological adaptation to stream-living (Liao & Lu, 2010).

Age and body size data were fitted to the von Bertalanffy (1957) model as follows:

$$SVL_t = SVL_{\max} - (SVL_{\max} - SVL_0) e^{-kt+b}$$

where SVL_t is SVL at age t , SVL_{\max} the estimated asymptotic SVL, SVL_0 the mean SVL at metamorphosis [In this study, $SVL_0 = 36.5 \text{ mm} (\pm 0.4, n=4)$], and k the rate at which the asymptotic SVL is approached.

RESULTS

We obtained a skeletochronological age for 128 specimens (5 juveniles and 123 adults). Juveniles were estimated to be 2 ($n=3$) or 3 ($n=2$) years old. The youngest adult individuals were also 2 and 3 years old, and the oldest individuals were 9 and 10 years old in males and females, respectively. Mean adult age for males and females was 5.5 ± 0.3 years ($n=43$) and 6.3 ± 0.2 years ($n=80$), respectively. This difference was statistically significant (Student's t test, $t=2.40$, $df=121$, $p=0.018$; Table 1).

Males averaged 67.1 ± 1.0 mm in SVL (range 48.4–78.2 mm, $n=43$), and females averaged 77.8 ± 1.0 mm in SVL (range 44.9–97.4 mm, $n=80$), the former being significantly smaller than the latter ($t=6.89$, $df=121$, $p<0.001$). ANCOVAs with age as covariate showed that the sexual difference in body size remained significant when taking age into account ($F=59.47$, $df=1, 120$, $p<0.001$). Regardless of sex, there was a significant positive correlation between body size and age (estimated coefficient \pm SE = 3.67 ± 0.28 , $F=177.04$, $df=1, 120$, $P<0.001$).

Table 1. Body size of *Feirana taihangnicus* by sex and age.

Age (Year)	Males			Females		
	<i>n</i>	mean \pm SE	Range	<i>n</i>	mean \pm SE	Range
2	1	48.4	–			
3	6	57.9 \pm 0.7	56.1–61.2	3	57.3 \pm 6.3	44.9–65.6
4	7	63.2 \pm 1.7	54.7–67.2	8	68.4 \pm 1.0	64.2–72.9
5	9	68.5 \pm 1.1	64.5–73.7	14	70.9 \pm 1.9	56.6–80.6
6	7	68.8 \pm 1.3	64.8–75.2	22	80.1 \pm 1.0	70.0–89.2
7	6	71.0 \pm 1.2	67.0–75.2	15	80.0 \pm 1.5	65.6–89.8
8	3	74.9 \pm 0.7	73.8–76.2	7	84.8 \pm 1.6	80.6–90.1
9	4	74.6 \pm 1.8	70.5–78.2	10	86.4 \pm 1.0	82.7–93.0
10				1	97.4	–

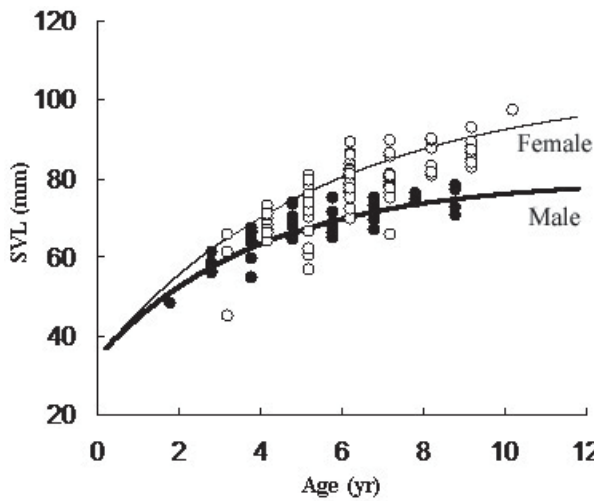


Fig. 2. Growth of body size of *Feirana taihangnicus*. The curves were produced by the von Bertalanffy model. male: $SVL_t = SVL_{max} - (SVL_{max} - 36.5)e^{-0.25t + 0.04}$; female: $SVL_t = SVL_{max} - (SVL_{max} - 36.5)e^{-0.16t + 0.03}$.

Body size of the three 2-year old juveniles was 45.2 ± 1.7 mm (42.1–47.8 mm), smaller than that of one adult of the same age (48.4 mm). This was also the case for 3-year old individuals (juvenile: 50.5 ± 1.4 mm, 49.1–51.8 mm, $n=2$; adult: 57.7 ± 1.9 mm, 44.9–65.6 mm, $n=9$). Body size of both male and female frogs increased following the von Bertalanffy model (Table 2; Fig. 2). Males were smaller in body size throughout their life, had a smaller asymptotic size and grew at a faster rate than females.

DISCUSSION

Females of *F. taihangnicus* were larger than males, a pattern followed by most anuran species (Shine, 1979). A positive correlation between female body size and fecundity (Chen et al., 2011) suggests that this female-biased size dimorphism might have evolved through fecundity selection (Pincheira-Donoso & Tregenza, 2011). Moreover, females of this species matured later, grew at a slower rate and attained older ages than males. Therefore, larger body size in females relative to males should be due to a longer lifespan (Liao & Lu, 2012), in agreement with what was found across other anuran species (Monnet & Cherry, 2002; Zhang & Lu, 2013b).

We previously showed that *F. taihangnicus* larvae spend 27 months and experience two winters in streams before metamorphosing into juveniles. In this study, we observed juveniles at an age of 2–3 years. Histological

Table 2. Growth parameters (mean \pm SE) estimated by the von Bertalanffy model [$SVL_t = SVL_{max} - (SVL_{max} - SVL_0)e^{-kt+b}$] for *Feirana taihangnicus*.

	Males	Females
SVL_0 (mm)	36.5	36.5
SVL_{max} (mm)	80.0 ± 3.0	104.1 ± 8.4
k	0.25 ± 0.04	0.16 ± 0.04
b	0.04 ± 0.07	0.03 ± 0.08
r^2	0.84	0.70

sections of bones for *Nanorana parkeri* tadpoles failed to find any LAGs probably because the tadpole bones remain unossified (Ma et al., 2009). Given that the minimum adult males and females were 2 and 3 years old, respectively, the frogs need at least five or six years from eggs to first reproduction.

Compared to arboreal and terrestrial species, stream-dwelling anurans are characterized by delayed maturation, slow growth and long lifespan. For example, the mean age of stream-living species is more than 60% and 25% greater than that of arboreal and terrestrial species of similar body size (our unpublished data). This slow life history corresponds with the fact that stream-breeding anurans, including *F. taihangnicus* (Chen et al., 2011), lay smaller clutches with larger eggs which take longer to finish metamorphosis (Summers et al., 2007). Stream habitats are mostly restricted to high elevations where climates are colder, food resources poorer and growing seasons shorter. Larger eggs and larvae are advantageous for surviving in harsh environments, where total investment in annual reproduction is limited (Kaplan & King, 1997). Harsher conditions may also constrain the growth potential of juvenile and adult frogs, which in turn will prolong their age at first reproduction and extend their longevity (Dmitriew, 2011; Zhang & Lu, 2012).

Many anuran species throughout the world have suffered a dramatic population decline during the last two decades, and the decline has most severely affected taxa living in stream habitats at high elevation (Mahony, 1996; Lips et al., 2003; Hero et al., 2005). Independent of phylogeny, the probability of population declines may be associated with large body size (Lips et al., 2003), small clutch size (Hero et al., 2005) and slower life histories which result in longer generation times and thus low population turnover rates (Purvis et al., 2000). *Feirana taihangnicus* follows a slow life history in terms of clutch size, egg size, tadpole and juvenile development and lifespan (Chen et al., 2011; this study). Although we have no historical information to ascertain whether *F. taihangnicus* populations have declined, evidence shows that the frogs are highly vulnerable to habitat alteration (Chen et al., 2011), and highlights an urgent need for protection measures for this high-elevation species.

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