SALAMANDRA **57**(2): 291–294
15 May 2021 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Correspondence

Growth trajectory of the world's largest aquatic frog (*Telmatobius macrostomus*): skeletochronological analysis of digit growth marks

Ulrich Sinsch[1] & César Aguilar-Puntriano[2]

[1)] Universität Koblenz-Landau, Institut für Integrierte Naturwissenschaften, Abteilung Biologie, Universitätsstr. 1, 56070 Koblenz, Germany

[2)] Universidad Nacional Mayor de San Marcos, Museo de Historia Natural de San Marcos, Departamento de Herpetología, Av. Arenales 1256, Lima 11, Peru

Corresponding author: Ulrich Sinsch, e-mail: sinsch@uni-koblenz.de

Manuscript received: 8 February 2021
Accepted: 17 February 2021 by Stefan Lötters

The Lake Junín Frog *Telmatobius macrostomus* (Peters, 1873) is a fully aquatic inhabitant of Central Peruvian lakes at elevations of 3,200–4,600 m above sea level (Vellard 1951, Sinsch 1986, Watson et al. 2017a; Fig. 1A). Fjeldså (1983) stated that the largest specimen of the scientific museum collection in Ondores (village at the shore of the Junín Lake) had a snout–vent length (SVL) of 30 cm, hind limbs of about 70 cm and a body mass of 2.8 kg, with collection date unknown. Sinsch (1986) reported a preserved specimen of 28.5 cm in the private collection of F. Tueros, Ondores. These data place *T. macrostomus* close to the largest extant frog species, the semiaquatic Afrotropical Goliath frog, *Conraua goliath*, reaching a SVL of 34 cm and a body mass of about 3.3 kg (Sabater-Pi 1985). Currently, the IUCN Red List of Threatened Species classifies this species as "endangered" and populations are considered declining due to water pollution and human consumption (Angulo 2008, Aguilar-Puntriano et al. 2010, Watson et al. 2017a, IUCN SSC Amphibian Specialist Group 2018).

Beginning in the 1990s, populations of *T. macrostomus* have declined dramatically (Arias-Segura 2003, Palacios-Zamudio 2020). To the best of our knowledge, large specimens (SVL > 20 cm) have not been collected during the past four decades. Frogs commercialized in the local food markets are mostly in a SVL range of 10–17 cm and rarely reach 20 cm (e.g. Ayala-Ascencio 1977, Sinsch et al. 1995, Castillo-Paredes 2008). This raises the question, whether large-sized individuals have become rare to absent because gaining large size requires an extended longevity in this species, i.e., the chance of enjoying a long lifespan might have decreased in response to environmental threats (Angulo 2008, Aguilar-Puntriano et al. 2010). The hypothesis that in unaltered habitats large *T. macrostomus* are long-lived, seems plausible because (1) amphibians grow indeterminately and (2) adult *T. macrostomus* as top-predator of the Andean lake ecosystem do not suffer from non-human predation (Ayala-Ascencio 1977, Harihan et al 2016, Watson et al. 2017b). If this hypothesis holds true, informed conservation management of these endangered frogs increases in difficulty. In this pilot study we aimed to establish the size–age relationship in these enigmatic frogs, using skeletochronology (Sinsch 2015).

The sample analysed included 31 individuals: ten preserved specimens housed in the collection of the Museo de Historia Natural de San Marcos (MUSM) in Lima; 21 unpreserved specimens collected by local fishermen for food supply in Lake Junín. Geographic origin and collection date of the samples were the lakes Yuncupuquio (n = 2, col. July 1988, MUSM 11077–11078), Paca (n = 2, col. August 1956, MUSM 376, 429), Capillacocha (n = 5, col. May 1949, MUSM 3, 4, 8, 424–425), and Chinchaycocha (= Junín Lake, n = 1, col. November 1947, MUSM 335, n = 21, col. March 1988). Each individual was sexed (presence of nuptial pads in males, eggs in the ovaries of the females) and SVL (defined as the distance between snout tip and cloaca) measured to the nearest 0.5 mm using a calliper. For skeletochronological age determination the 3rd or 4th digit of the right forelimb was toe-clipped and stored individually in 70% ethanol at room temperature until histological examination. Laboratory protocols followed the standard methods of skeletochronology (Sinsch 2015, Sinsch & Dehling 2017). The samples were embedded in Historesin™ (JUNG) and stained with 0.5% Cresyl violet. Diaphysis was cross-sectioned at 12 µm using a JUNG RM2055 rotation microtome. Cross sections were examined light microscopically for the presence of growth marks at magnifications of 400× using an OLYMPUS BX 50. We distinguished strongly stained lines of arrested growth (LAGs, annual growth marks) in the periosteal bone, separated by faintly stained broad growth zones, and the line of metamorphosis (LM), separating larval from postmetamorphic bone. We selected diaphysis sections in which the size of the medullar cavity was at its minimum and that of periosteal bone at its maximum. The number of LAGs represents the number of years completed.

Growth following metamorphosis was estimated using the equation $SVL_t = SVL_{max} - (SVL_{max} - SVL_{met}) \times e^{-k \times t}$ (von Bertalanffy 1938). SVL_t is the average body length at age t, SVL_{max} the asymptotic maximum body length, SVL_{met} the body length at metamorphosis, t the number of growing seasons experienced (n LAGs), and k is the growth coefficient (i.e., slope of the growth curve). SVL_{met} ranged from 67–75 mm (arithmetic mean: 70 mm) in the metamorphic stages of tadpoles collected in the field (Rodriguez-Papuico 1974, Sinsch 1986; Fig. 1B). The von Bertalanffy growth model was fitted to the average growth curve using the least square procedure (nonlinear regression, Statgraphics Centurion 18, version 18.1.13). Estimates of SVL_{max} and k are given with the corresponding 95% confidence interval.

Age determination was successful in all individuals. LAGs were resolved as dark stained, more or less sharp lines contrasting from the faint violet background colouration of the periosteal bone (Fig. 2). In some subadult individuals the line of metamorphosis was still discernible (Fig. 2A, B). Double line formation was common in many individuals, but easily distinguishable from non-periodical faint growth marks caused by the alternation of periods with fast and slow growth within the same annual growth period (Fig. 2C, D). Frogs with a SVL less than 100 mm (n = 9) were subadults captured between metamorphosis and the formation of the first LAG (Fig. 3). Sexual maturation (nuptial pads in males, egg formation in females) required a minimum SVL from 118–120 mm. Consequently, only one out of the six individuals showing one LAG was an adult female. Adults examined had 2–6 LAGs (except the mentioned female), with two females being the largest and oldest individuals of the sample (Fig. 3). The von Bertalanffy growth model for the pooled data set indicated a SVL_{max} of 156 mm ($CI_{95\%}$: 138–174 mm) and a growth coefficient k of 0.59 ($CI_{95\%}$: 0.33–0.85).

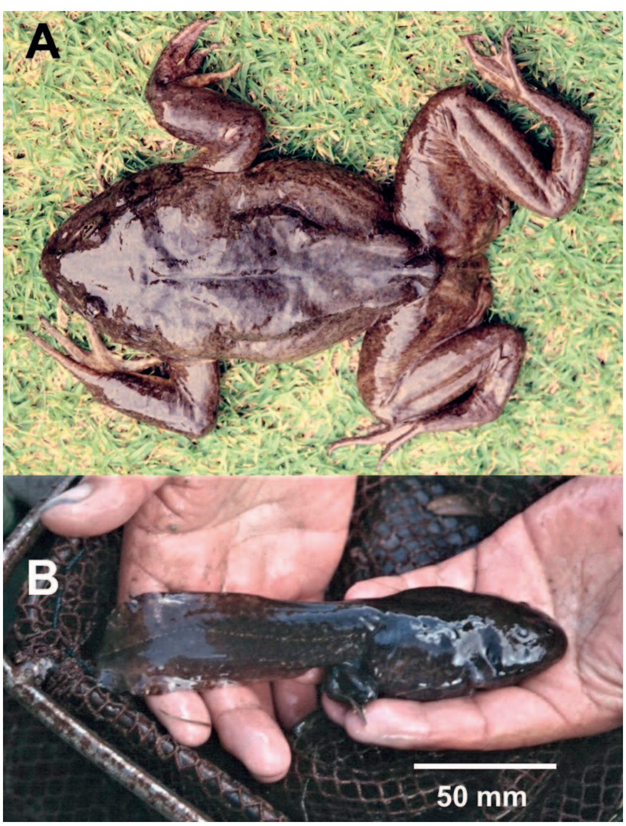

Figure 1. *Telmatobius macrostomus*: (A) Adult female, SVL 159 mm, collected in the Laguna Punrún, Cerro de Pasco, March 1984; (B) metamorphic tadpole, Gosner stage 41, body length 75 mm, total length 179 mm, collected in the Criadero experimental de Rana, Huancayo, Junín, March 1988.

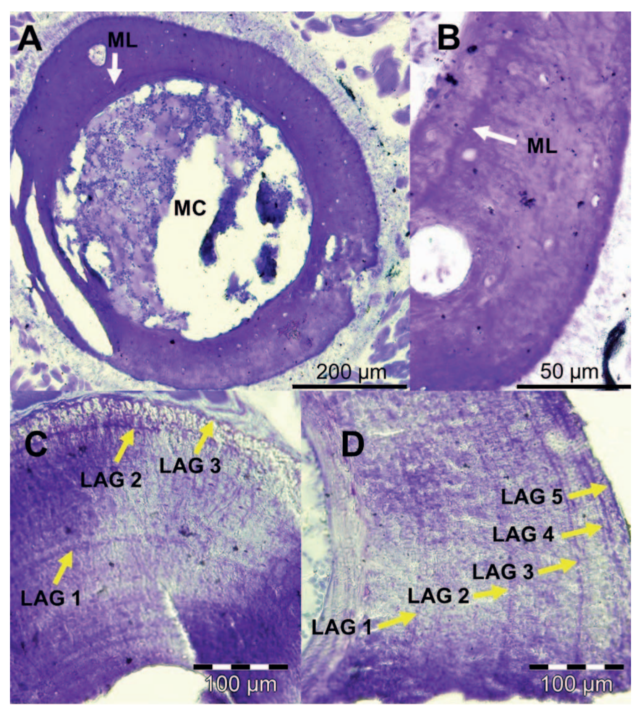

Figure 2. Stained cross-sections of *Telmatobius macrostomus* phalange bones: (A, B) Juvenile, SVL 69.5 mm, 0 LAGs, metamorphic line (ML) visible, collected in the Laguna Capillacocha, Junín, May 1949, MUSM 4; (C) male, SVL length 123 mm, 3 LAGs, collected in the Laguna Chinchaycocha, Junín, November 1947, MUSM 335; (D) female, SVL length 155 mm, 5 LAGs, collected in the Laguna de Paca, Junín, August 1956, MUSM 429. MC means medullar cavity.

Skeletochronology again proved to be a useful method for the age estimation in tropical frogs without marked seasonal reinforcement of the circannual growth rhythm, the ultimate cause of LAG formation (Castanet 1993, Sinsch 2015, Sinsch & Dehling 2017). Annual fluctuation of average water temperature in Lake Chinchaycocha is regular with its minimum in July (ca. 4.5–5.8°C) and its maximum in March (up to 8°C; Castillo-Paredes 2008). Thus, temperature variation may contribute to reinforce the circannual rhythm. Yet, realized longevity of *T. macrostomus* examined exceeds probably the number of LAGs because they do not reflect the period of larval development. Studies on captive grown tadpoles (Centro Piloto de Experimentación y Producción Acuícola "Arroyo Verde", Ninacaca, Lake Junín) suggest that 15–19 months are needed to complete metamorphosis (Vásquez 1953, Rodriguez-Papuico 1974, Alt & Pnud 2002). As we analysed the digits of the forelimb for age estimation, we missed probably the first year of tadpole life because the digits develop late in tadpole growth. In captivity, all juveniles reached sexual maturity 30 months after metamorphosis, but mostly at an age of two years (Alt & Pnud 2002). Our skeletochronological data indicate that in the natural habitat sexual maturation occurs later, rarely after two years, generally after three. The discrepancy of about one year between captive grown and wild individuals may be due to the optimized nutrition and water temperatures during captivity.

The scarce data available on growth of captive *T. macrostomus* emphasize that skeletochronology did not grossly misjudge the demography of free-ranging individuals. Thus, the oldest specimens of the samples were 6–8 years old, which is in the same magnitude as the similar-sized mountain chicken *Leptodactylus fallax* in the West Indies (Guarino et al. 2014). The maximum size of 20 cm at most recorded during the past decades might be related to the local extinction of the preferred fish prey *Orestias* spp. due to water pollution by mining residuals (Beccara-Diaz 2012). The present prey items include almost exclusively

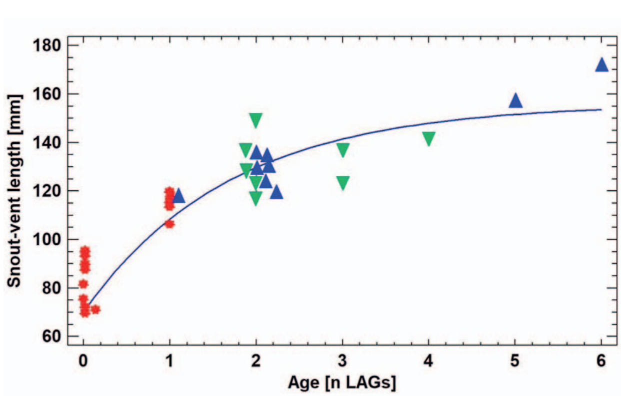

Figure 3. Von Bertalanffy growth model of age–size relationship in post-metamorphic *Telmatobius macrostomus*. Every dot represents an individual frog, subadult = red asterisk, male = green triangle, female = blue triangle. For statistical details see text.

macrozoobenthos organisms, presumably a low-quality nutrition compared with that consisting of fish (Watson et al. 2017a). Since the greatest portion of adult size is gained during the juvenile period, in which fish prey may provide more resources for growth than snails and gammarids, the age size-relationship established in Figure 3 is probably valid only for growth in suboptimal conditions (low-quality food, polluted water). The record of very large frog specimens in the last century may indicate that formerly habitat conditions were superior to presently allowing for an increased growth coefficient k and for a prolonged adult growth period. Realized maximum lifespan under these conditions exceeded probably eight years by far. We conclude that an informed conservation management of these emblematic frogs of the high Andes should ban the traditional hunting for food supply (killing actually mainly juveniles and young adults) and attempt to improve the state of the lakes inhabited (reduce pollution, erase the introduced rainbow trout).

Acknowledgements

We thank Brigitte Nilow-Lange for her skilled assistance during the laboratory procedures.

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