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Review: Skeletochronological assessment of demographic life-history traits in amphibians

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A long-standing challenge for amphibian population ecologists is the reliable estimation of age in individuals without known recapture history. The development and widespread application of skeletochronology seems to have solved the issue, although studies evaluating the precision and reliability of age estimates are rare and controversial. Skeletochronology attempts to relate the number of dense hematoxylinophilic narrow growth marks (Lines of Arrested Growth, LAGs) separated by faintly stained broad growth zones in the round bones of Anura and Caudata with age. In this review, I briefly summarise the laboratory procedures to obtain informative histological cross sections, and the interpretation of growth marks becoming visible through staining. I discuss the precision and constraints of skeletochronological age estimation by evaluating evidence derived from individuals with known age. Individuals of up to eight years of age are correctly aged by LAG-counting, whereas the lifespan of older individuals is systematically underestimated, due to the increasing rapprochement of LAGs at the periphery of bones. A case study on the latitudinal and altitudinal variation of demographic life-history traits in natterjack toads illustrates the utility of skeletochronology to assess plasticity of age distributions in response to climate variation. Finally, I provide perspectives for future research to which skeletochronology may contribute valuable information.

Key words: age at maturity, growth pattern, large-scale variation among and within species, life history, longevity, skeletochronology

INTRODUCTION

The analysis of amphibian demography and its variation among populations or species requires information on age and growth patterns of a large number of specimens. The most reliable, but extraordinarily labour-intensive method to obtain an age-size relationship is capturing, marking and releasing (CMR) a large number of individuals of known age (ideally metamorphs) before recapturing them at regular intervals (e.g., Durham & Bennett, 1963; Wagner et al., 2011). However, CMR-studies do not solve the problem of aging unmarked individuals with unknown growth history (e.g., Turner, 1962; Alcobendas & Castanet, 2000; Leskovar et al., 2006). Attempts to translate body length-frequency into age classes (e.g., Bannikov, 1950; Tamsitt, 1962; Loman, 1978; Semlitsch, 1980; Schnäbele, 1987) fail because growth of amphibians slows down after attaining sexual maturity, and the size of adults mainly depends on the size attained during the subadult growth period (e.g., Turner, 1960; Halliday & Verrell, 1988; Smirina, 1994; Sinsch et al., 2010). Similarly, the supposed relationship between number of testis lobes and age in caudate amphibians (Humphrey, 1922) proved unreliable (Tilley, 1977; Dolmen, 1982; Verrell et al., 1986).

During the past decades, reconstruction of the growth history by skeletochronology, i.e. the use of incremental growth marks stained in bone cross sections, has become a standard procedure to estimate the age of amphibians (Castanet, 2002), squamates (Buffrénil & Castanet, 2000), dinosaurs (Horner et al., 1999), birds (Bourdon et al., 2009) and mammals (Marin-Moratalla et al., 2013). The first successful attempts on amphibians were based on skull bones (parasphenoid *Necturus maculosus*, Senning, 1940; pterygoid *Lithobates [Rana] catesbeianus*, Schroeder & Baskett, 1968), after which round bones have become the material of choice (Kleinenberg & Smirina, 1969; Smirina, 1972a, b; Castanet & Smirina, 1990; Castanet et al., 1993). The use of long bones such as humerus and femur required unacceptably large numbers of individuals being sacrificed, and non-destructive age determination became available since Smirina (1972a) demonstrated that phalanges obtained by toe-clipping provided the same information.

By now, several hundreds of skeletochronological studies on amphibians have been published, mainly focusing on the population ecology of temperate-zone Anura and Caudata while very few dealt with Gymnophiona and tropical amphibians. This review aims to give a state-of-the-art summary of the accumulated knowledge

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obtained with this method, critically highlighting its reliability and constraints and proposing future research avenues.

Detection and interpretation of growth marks in periosteal bone

Because the periosteal cortex is broadest and the medullar cavity narrowest in the middle part of the diaphysis of limb bones, this section is best-suited for skeletochronological detection of growth marks (Castanet & Smirina, 1990; Castanet et al., 1993). If phalanges obtained by toe-clipping are used, the basal and middle phalanges provide better resolution than the most distal phalange. In *Gymnophiona*, trunk vertebrae are the material of choice (Measey & Wilkinson, 1998; Scholz et al., 2010). Material may be stored as dry clean bone or preserved in 70% ethanol or 4% formaldehyde at room temperature. Standard laboratory procedures of bones include decalcification (with 3–5% nitric or formic acid), fixation (with Bouin's solution), and embedding (paraffin, Histoiresin™ or water for freezing microtomes; Smirina, 1972a, b; Hemelaar & van Gelder, 1980; Miaud et al., 1993; Sinsch et al., 2001). Embedded bones are cross-sectioned at usually 10–16 µm, although Wagner et al. (2011) state that reducing sections to 7 µm improves the distinction between growth marks of very old individuals. Sections are stained with Ehrlich's, Delafield's or Mayer's haematoxylin, acridine orange, cresylviolet or similar dyes until growth marks become visible (e.g., Smirina, 1972a, b; Juarranz & Paton, 1990; Sinsch et al. 2001). Microscopic examination of at least 10 cross sections per diaphysis by two independent observers is recommended, because not every section provides the complete information. The most suitable cross sections are characterised by a minimum size of the medullar cavity, and a maximum size of (periosteal) bone. In addition to direct observation, digital images including overlay and other optimising procedures for further analysis of individual growth pattern can be used (e.g., Sinsch et al., 2007a; Wagner et al., 2011).

The histological interpretation of suitable phalanx diaphysis sections for anuran amphibians inhabiting a temperate, a Mediterranean and a tropical climate is exemplified in Fig. 1. The central part of a diaphysis section is the medullar cavity, sometimes filled with cartilage (in young juveniles) or with endosteal bone (in old specimens; EB). At the periphery of the medullar cavity, endosteal resorption can replace part of the inner periosteal bone including growth marks (Castanet, 1975; Hemelaar, 1985; Castanet & Smirina, 1990; Castanet et al., 2000). The limit between endosteal and periosteal bone often stains hematoxylinophilic representing the resorption line (Hemelaar, 1981). While older individuals tend to accumulate more endosteal bone than younger ones, the proximate causes of endosteal resorption are not well understood. In some populations of *Bufo bufo*, for example, resorption is significant in most individuals, whereas in other populations it does not play a major role (e.g., Smirina 1972b, 1983; Hemelaar, 1981, 1985; Kuhn, 1994). Specifically in young individuals and in those with little endosteal resorption, the first discernible growth

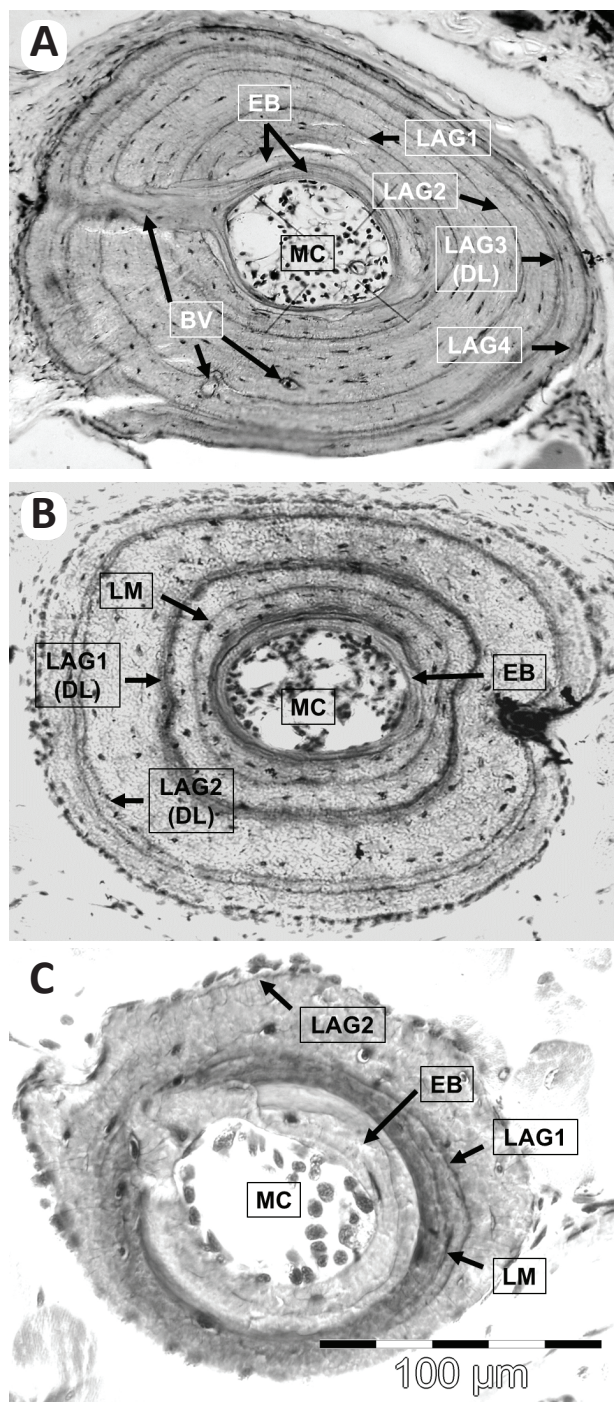


Fig. 1. Examples of phalanx cross sections and their histological interpretation. (A) *Pseudepidalea [Bufo] viridis* female, SVL 66mm, 4 LAGs captured in June 2000 at Urmitz (50°21'43" N, 7°35'48" E, 60m asl; photo: Sinsch & Leskovar); (B) *Pseudepidalea variabilis [Bufo viridis]* juvenile, SVL 50mm, 2 LAGs captured in March 2005 at Herzliyya (32°10'01" N, 34°49'35" E, 28m asl; photo: Sinsch & Elron); (C) *Hyperolius castaneus* male, SVL 24mm, 2 LAGs, captured in October 2010 in the Nyungwe National Park (2°30'12" S, 29°11'50"E, 2,379m asl; Photo: Sinsch). Abbreviations: BV=blood vessel, DL=double line, EB=endosteal bone, LAG=line of arrested growth, LM=line of metamorphosis, MC=medullar cavity.

mark is often the faint line of metamorphosis (LM; Fig. 1B, C). Between the LM and the outer periphery of the periosteal bone, faintly stained broad growth zones

(fast osteogenesis) consisting of parallel or woven-fibered bone and narrow, alternate with strongly stained hematoxylinophilic lines (LAGs, lines of arrested growth, Fig. 1; Castanet et al., 1993). LAG formation can be induced experimentally by several weeks of cold or starvation (Smirina et al., 1986). However, the ultimate cause of annual LAG formation is a genetically based, circannual rhythm synchronised with seasonal cycles (Castanet et al., 1993). Therefore, LAGs are also formed in tropical habitats with very little seasonality (e.g., Fig. 1C; Khonsue et al., 2000). Besides LAGs, diaphysis sections sometimes show broad, faintly stained annuli (Peabody, 1961; Castanet et al., 1993; Alcobendas & Castanet, 2000) within long-lasting growth periods, indicating reduced but not arrested growth (LRG, Sinsch et al., 2007a). Irregularities such as multiple LAGs and LRG may complicate age estimation, and can represent histological equivalents of interrupted hibernation during periods of elevated ambient temperature (multiple LAGs, Fig. 1A, B; e.g., Sinsch et al., 2007a), growth arrest during hibernation and estivation (e.g., Olgun et al., 2005), or reduced growth during estivation (e.g., Jakob et al., 2002; Leclair et al., 2005).

Individual growth patterns are reconstructed from diaphysis sections using LAG/bone diameter (BM) or the area between two neighbouring LAGs as a one- or two-dimensional estimate for magnitude of age-dependent body size increment (e.g., Hemelaar, 1988; Alcobendas & Castanet, 2000; Caetano & Leclair, 1996; Homan et al., 2003; Olgun et al., 2005; Sinsch et al., 2007a). In the newt *Notophthalmus viridescens*, humerus perimeter correlated significantly with snout-vent length (SVL, Caetano & Leclair, 1996). The accuracy of eight back-calculation formulae (Francis, 1990) used to reconstruct amphibian SVL from LAG/bone diameter was evaluated by Marunouchi et al. (2000) in a combined CMR and skeletochronological study on *Rana japonica*. All formulae tended to overestimate the actual age-dependent SVL, and only the Dahl-Lea

method ($SVL[mm] = SVL_{at\ capture}[mm] * BM_{at\ ith\ LAG}[\mu m] / BM_{at\ capture}[\mu m]$) resulted in non-significant deviations. In contrast, the two-dimensional approach using the area between two LAGs proved to be a valuable tool to analyse the sex-specific and inter-population variability of growth pattern in *Ambystoma maculatum* (Homan et al., 2003) and in *Epidalea [Bufo] calamita* (Sinsch et al., 2010).

Precision and constraints of age determination

The correspondence of LAGs with the repetition of the annual cycle has been verified in CMR studies or by vital dye injections depositing time marks in the bone tissue (e.g., Schroeder & Baskett, 1968; Smirina, 1972a; Francillon, 1979; Hemelaar & van Gelder, 1980; Smirina & Makarov, 1987; Böll et al., 1997; Tejedo et al., 1997; Eden et al., 2007; Matsuki & Matsui, 2009; Wagner et al., 2011). The results of most but not all studies confirmed the formation of one LAG per year in Palaearctic and subtropical species (Fig. 2). In anurans and urodeles with a known age of maximum seven years, the median rate of correct skeletochronological age estimates is 86.2% (range: 29–100%; Fig. 3). Deviations from the actual age were almost generally caused by underestimation (mostly 1–2 years), and rarely by overestimation (1 year in *Hynobius nebulosus*; Matsuki & Matsui, 2009). Underestimation can be caused by unrecognised resorption of the inner LAG(s) or by rapprochement of peripheral LAGs after attaining sexual maturity, overestimation by unrecognised double lines or by confusion of LM with LAG (e.g., Hemelaar, 1985; Castanet & Smirina, 1990; Smirina, 1994). Thus, calibration of histological age estimation with known-age individuals confirms that the use of skeletochronology is precise enough for demographic studies, if individuals of less than 8 years form the dominant age fraction of the populations (except for Böll et al., 1997). This is surely true for most temperate zone and tropical anurans inhabiting low altitudes (e.g., Miaud et al., 1999; Morrison et al.,

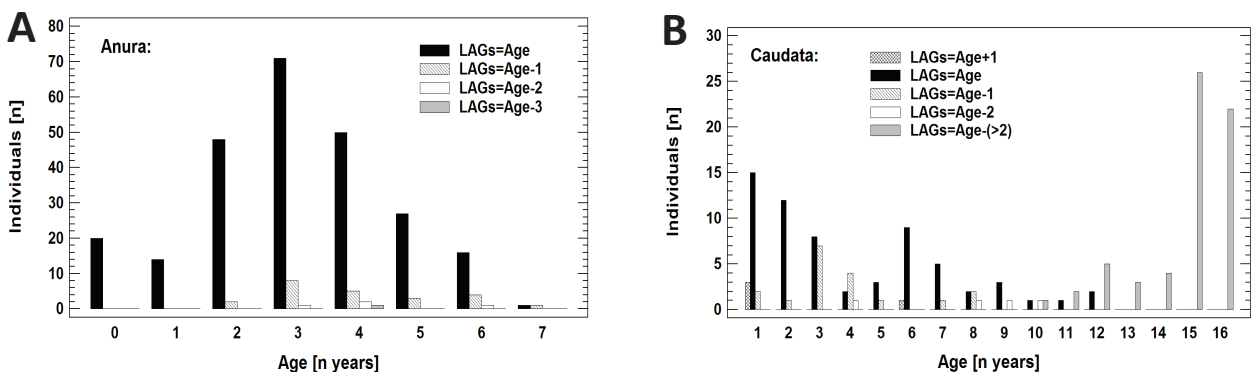


Fig. 2. Actual and skeletochronological age estimates in Anura (A) and Caudata (B). Data on 275 known-age anuran and on 152 known-age urodeles were compiled from Schroeder & Baskett (1968; 42 *Lithobates [Rana] catesbeianus*), Kalb & Zug (1990; 2 *Anaxyrus [Bufo] americanus*), Driscoll (1999; 8 *Geocrinia alba*, 20 *G. vitellina*), Francillon & Castanet (1985) and Embrechts & Reyer (2012; 6+20 *Pelophylax [Rana] esculentus*), Hemelaar & van Gelder (1980; 100 *Bufo bufo*), Tejedo et al. (1997; 7 *Epidalea [Bufo] calamita*); Böll et al. (1997, 12 *Alytes obstetricans*); Marunouchi et al. (2000; 13 *Rana japonica*), Smirina (1972a) and Gibbons & McCarthy (1983; 9+21 *Rana temporaria*); Eden et al. (2007; 37 *Ambystoma tigrinum*), Francillon (1979; 8 *Triturus cristatus*), Caetano & Castanet (1987; 3 *Triturus marmoratus*), Wagner et al. (2011; 45 *Ichthyosaura [Triturus] alpestris*); Ento & Matsui (2002) and Matsuki & Matsui (2009; 11+48 *Hynobius nebulosus*).

Table 1. Longevity and median age in some representative amphibian populations along the latitudinal gradient.

Species	Latitude	Altitude [m a.s.l.]	Median Age [LAGs]	Longevity [LAGs]	Reference
<i>Rana temporaria</i>	69° N	480	12	18	Patrelle et al., 2012
<i>Rana temporaria</i>	63° N	5	5	8	Hjernquist et al., 2012
<i>Pelophylax ridibundus</i>	51° N	110	4	7	Socha & Ogielska, 2010
<i>Salamandrella keyserlingii</i>	51° N	1560	7	12	Hasumi & Borkin, 2012
<i>Pseudepidalea viridis</i>	50° N	60	3	6	Sinsch et al., 2007b
<i>Hyla arborea</i>	50° N	293	2	4	Nemnich et al., 2005
<i>Crinia signifera</i>	43° S	897	3	7	Lauck, 2005
<i>Pleurodema thaul</i>	43° S	20	3	5	Iturra-Cid et al., 2010
<i>Hyla orientalis</i>	41° N	800	5	8	Altunışık & Özdemir, 2013
<i>Lissotriton vulgaris</i>	40° N	32	3	5	Altunışık et al., 2014
<i>Feirana taihangnicus</i>	35° N	760	6	10	Zhang et al., 2013
<i>Amietophrynus pardalis</i>	34° S	76	3	6	Cherry & Francillon-Viellot, 1992
<i>Hyla annectans</i>	30° N	800	2	4	Liao & Lu, 2010
<i>Litoria chloris</i>	28° S	160	4	6	Morrison et al., 2004
<i>Argentohyla siemersi</i>	27° S	62	4	5	Cajade et al., 2013
<i>Rana swinhoana</i>	25° N	200	4	7	Lai et al., 2005
<i>Pyxicephalus adspersus</i>	25° S	1398	6	16	Yetman et al., 2012
<i>Polypedates maculatus</i>	20° S	56	3	6	Mahapatra et al., 2008
<i>Dyscophus antongilii</i>	15° S	448	6	11	Tessa et al., 2007
<i>Hylarana nigrovittata</i>	13° N	90	2	7	Khonsue et al., 2000
<i>Nimbaphrynoides occidentalis</i>	8° N	1752	1	5	Castanet et al., 2000
<i>Geotrypetes seraphini</i>	4° N	800	1	4	Scholz et al., 2010

2004; Leskovar et al., 2006; Sinsch et al., 2010; Tables 1, 2). Caution is recommended if populations are from high latitudes or altitudes, which may increase the longevity of amphibians (e.g., Morrison & Hero, 2003; Hjernquist et al., 2012; Oromi et al., 2012; Zhang & Lu; 2012; Tables 1, 2).

The percentage of correctly aged amphibians drops to zero at an actual age of 13 years in all species studied so far (Figs. 2B, 3). Actual age was always underestimated by at least three years, sometimes by up to 10 years in *Ambystoma tigrinum* (Eden et al., 2007), 13 years in *Epidalea [Bufo] calamita* (Sinsch et al., 2006), and 19 years in *Ichthyosaura alpestris* (Wagner et al., 2011). In long-lived amphibians, bone growth of elder individuals decreases so strongly that growth zones are too small to separate peripheral LAGs or LAGs may be completely absent (e.g., Alcobendas & Castanet, 2000; Sinsch et al., 2010). The reason for the decreasing precision of age estimates in elder individuals is that the paradigm of indeterminate growth in amphibians (e.g., Tilley, 1973; Sebens, 1987) does not hold true for their bones. Despite differences between species (Eden et al., 2007, Matsuki & Matsui, 2009), the evidence is overwhelming that the one LAG per year rule is not valid for old individuals.

A case study: metaanalysis of latitudinal and altitudinal variation of demographic life-history traits in the natterjack toad *Epidalea [Bufo] calamita*

An amphibian’s life history is a complex pattern of growth, development, storage and reproduction. Specifying general biogeographic patterns for amphibians, Morrison & Hero (2003) concluded that adults tend to be larger (Bergmann cline) and reach maturity at older ages at higher latitudes and altitudes. Recently, Zhang & Lu (2012) found in a meta-analysis on two urodele and nine anuran species that longevity increased at higher altitudes, but not at higher latitudes. An excellent test case for these generalised predictions is the natterjack toad *Epidalea [Bufo] calamita*, probably one of the best studied temperate-zone amphibians with respect to demography. To date, 20 populations throughout its entire geographic and altitudinal range have been subject to skeletochronological assessment of key parameters such as age at maturity (AM), longevity and potential reproductive lifespan (PRLS; references and detailed information listed in Table 2), allowing for a comprehensive meta-analysis of the proximate factors of life-history variation.

Table 2. Latitudinal and altitudinal variation of demographic life history traits in *Epidalea [Bufo] calamita* throughout most of its geographical range. AM=age at maturity; SVL=snout-vent-length; PRLS=Potential reproductive lifespan.

Locality	Coordinates	Altitude [m a.s.l.]	Sex	AM [LAGs]	Median Age [year]	SVL [mm; mean±SE]	Longevity [LAGs]	PRLS [year]	Reference
Dunnerholme (UK)	54°12' N 3°12' W	3	M, F	3 3	5 4	?	9 8	6 5	Denton & Beebee, 1993
Birkdale (UK)	53°36' N 2°59' W	8	M, F	2 3	3 5	?	7 9	5 6	Denton & Beebee, 1993
Dingle Peninsula (Ireland)	52°08' N 10°19' W	11	M, F	2	2	57	7	5	Gibbons & McCarthy, 1983
Halle (Germany)	51°29' N 11°58' E	96	M, F	2	3	55.6	7	5	Große, 1999
Woolmer (UK)	51°05' N 0°53' W	84	M, F	2 3	4 5	?	7 17	5 14	Banks et al., 1993
St. Augustin (Germany)	50°46'23''N 7°08'53''E	52	M, F	2 2	3 3	59.9 59.5	7 7	5 5	Sinsch, 1998
Urmitz (Germany)	50°21'43''N 7°35'48''E	60	M, F	2 2	3 3	58.1 60.8	7 8	5 6	Leskovar et al., 2006
Karlsruhe (Germany)	49°00' N 8°23' E	112	M, F	2	4	61.9	7	5	Schnäbele, 1987
Palamós (Spain)	41°51'49''N 3°07'59''E	10	M, F	1 2	3 3	61.6±0.8 59.4±1.6	5 5	4 3	Oromi et al., 2012
Balaguer (Spain)	41°46'30''N 0°46'50''O	220	M, F	2 2	4 3	79.1±0.8 80.1±1.4	7 5	5 3	Leskovar et al., 2006
Mas de Melons (Spain)	41°30'50''N 0°43'30''E	240	M, F	2 3	4 4	79.0±0.7 85.5±2.7	8 7	6 4	Leskovar et al., 2006
Navaluenga (Spain)	40°24'57''N 4°40'47''W	752	M, F	2 2	4 3	63.5±1.0 66.1±1.2	6 5	4 3	Oromi et al., 2012
El Pinós (Spain)	38°22'44''N 0°58'40''W	760	M, F	2 3	3 4	78.6±1.3 83.0±1.4	4 8	2 5	Oromi et al., 2012
Dehesa del Barraco (Spain)	40°27'53''N 4°38'12''W	920	M, F	2 3	3 3	65.5±1.0 67.0±2.4	5 5	3 2	Oromi et al., 2012
La Cedrera (Spain)	40°30'33''N 4°39'16''W	1472	M, F	2 3	3 3	63.9±0.9 66.5±1.4	6 6	4 3	Oromi et al., 2012
Cavadores (Spain)	40°16'19''N 5°15'12''W	2100	M	2	6	62.6±0.9	9	7	Oromi et al., 2012
Navasomera (Spain)	40°15'07''N 5°15'49''W	2270	M, F	5 5	7 6	58.8±0.9 60.3±3.5	12 6	7 1	Oromi et al., 2012
Toba, Villaviciosa de Cordoba (Spain)	38°04' N 5°01' W	700	F	2	?	?	10	8	Tejedo, 2003
Navas (Spain)	37°47'28''N 6°04'41''W	420	M, F	1 2	2 3	65.8±1.0 70.6±1.7	3 8	2 6	Sinsch et al., 2010
Reserva Biologica de Donana (Spain)	37°00'44''N 6°30'13''W	24	M, F	1 1	3 2	55.7±1.2 51.2±1.4	6 4	5 3	Sinsch et al., 2010
Locality	Coordinates	Altitude [m a.s.l.]	Sex	AM [LAGs]	Evidenced longevity in captive [years]		Reference		
Krefeld (Germany)	51°20'44'' N 6°36'29'' W	37	M, F	2 2	24 28		Sinsch et al., 2006		

Longevity assessed in natural populations is only about a third of that recorded in captive individuals, i.e. with food *ad libitum* and predation risk absent (Sinsch et al., 2006). This is in agreement with the findings in several other species (Blanco & Sherman, 2005; De Magalhaes & Costa, 2009; Guarino et al., 2014). As there is no indication that food is a limiting resource for

natterjacks (e.g., Denton & Beebee, 1993; Leskovar et al., 2004), predation pressure is probably the major cause for realised longevity being far below potential longevity. Analysing the combined effects of latitude, longitude and altitude as covariates on the sex-specific demographic traits ($\log_{10}$ -transformed data) reveals that (i) longitude did not influence any of the life-history traits tested; (ii)

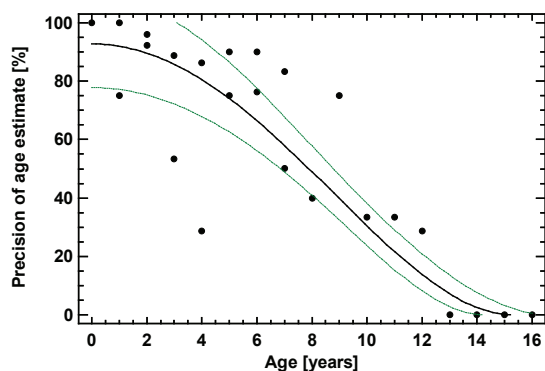


Fig. 3. Precision of skeletochronological age estimates per actual age class (anurans and urodeles separately). Percentage of correct assessment is based on the data presented in Fig. 2. Regression model: Precision=(9.63–0.041*Age²), $r^2=0.839$, $n=23$)

AM of males was significantly lower (about 0.5 years) than that of females (ANCOVA, $F_{1,37}=9.72$, $p=0.0038$), increasing significantly with latitude (ANCOVA, $F_{1,37}=23.31$, $p<0.00001$) and altitude (ANCOVA, $F_{1,37}=40.77$, $p<0.00001$); (iii) Longevity did not differ between sexes (ANCOVA, $F_{1,37}=0.77$, $p=0.38$) and increased again with latitude (ANCOVA, $F_{1,37}=13.27$, $p=0.0013$) and altitude (ANCOVA, $F_{1,37}=4.36$, $p=0.0445$); (iv) PRLS remained unaffected from geographical variation due to the parallel changes in AM and longevity (ANCOVA, $F_{1,37}=0.02$ – 3.66 , $p=0.0645$ – 0.8955); (v) age-adjusted SVL did not vary between sexes (ANCOVA, $F_{1,30}=13.27$, $p=0.61$), and increased with latitude (ANCOVA, $F_{1,30}=6.72$, $p=0.0157$) but not with altitude (ANCOVA, $F_{1,30}=0.70$, $p=0.41$).

This meta-analysis confirms the generalised prediction of Morrison & Hero (2003) that age at maturity increases along latitudinal and altitudinal clines, and is also in agreement with the conclusions drawn from smaller data sets of natterjack populations (Leskovar et al., 2006; Sinsch et al., 2010, 2012). However, age-adjusted size decreased with latitude and was unaffected by altitude, disagreeing with the prediction of Morrison & Hero (2003) and confirming a roughly converse Bergmann cline (Sinsch et al., 2010, 2012). The increase of longevity at higher latitudes also disagrees with the findings of Zhang & Lu (2012). This example demonstrates that multiple skeletochronological studies in the same species are needed for reliable conclusions about the proximate causes of geographical variation.

PERSPECTIVES

About five decades of experience with skeletochronology of amphibians have yielded demographic data on more than 200 species, predominantly anurans. The geographical bias towards temperate-zone species and the taxonomic bias towards anurans should motivate future studies on the neglected tropical species, including Gymnophiona. The few tropical species studied so far seem to be considerably shorter-lived than temperate-zone species (Table 1) suggesting rather distinct life-history constraints. It is currently unknown, whether

or not the fossorial lifestyle of most Gymnophiona affects their demography in a distinct way.

Avoiding pitfalls such as multiple lines and endosteal resorption of LAGs, the interpretation of periosteal growth marks provides precise age estimates for individuals of up to about eight years of age. The reliable reconstruction of age structure is thus feasible for most populations and species. There is a need for future research to distinguish natural geographical variation of demographic parameters from the effects of local anthropogenic habitat deterioration and climate change. Examples for the significant influence of local habitat quality on longevity are *Triturus cristatus* (Hagström, 1980; Miaud et al., 1993; Sinsch et al., 2003) and *Bufo [Bufo] viridis* (Sinsch et al., 2007b). Potential effects of climate change on local age structure are only detectable in long-term investigations on a set of local populations. Museum collections may help to reconstruct the age structure of amphibian populations decades or even a century ago. In conclusion, skeletochronology has the potential to contribute significantly to our knowledge on demography and life-history of amphibians in the future, provided that the described limits of reliability in old specimens are considered.

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