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Life history traits of a spadefood toad (*Pelobates cultripes*) population from a semiarid zone in the north east of the Iberian Peninsula

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Skeletochronology was used to estimate age and several life history traits of the Iberian spadefoot toad (*Pelobates cultripes*) from a semiarid zone of the Ebro Valley (in the northeastern Iberian Peninsula). The lines of arrested growth (LAGs) were clearly visible in all cross sections of the adult phalanges, showing fast growth in the first year of life, with large adult sizes. The growth, size (SVL) and age structure varied between sexes, suggesting that the larger size of females (mean±SE=76.14±0.95 mm; males: 71.76±0.90 mm) is related to a delay in age at maturity (3 years) compared to males (2 years). In addition, females were more long-lived (6 years) than males (5 years), resulting in a similar potential reproductive life span (PRLS=3 years). A data set including life history traits from several *P. cultripes* populations was used to analyse demographic variation. We suggest multiple factors such as altitude, latitude, predation, and soil characteristics can explain life history trait variation in this species.

Key words: Iberian spadefoot toad, lines of arrested growth, semiarid environment, skeletochronology

INTRODUCTION

Reproductive life history is one of the important evolutionary and ecological traits in amphibians. Life histories are known to vary geographically, and can be explained by regional differences in habitat quality, altitude and latitude, as well as climate (Morrison & Hero, 2003; Sinsch et al., 2010; Oromi et al., 2012). Age estimation allows the analysis of variation in life history traits such as growth rates, longevity, age structure and age at sexual maturity among populations. Skeletochronology is a standard procedure to estimate the age of amphibians and is based on the presence of lines of arrested growth (LAGs), formed in the periosteal part of the bones when growth is interrupted by hibernation or aestivation (Guarino et al., 1998; Castanet, 2002; Leclair et al., 2005; Olgun et al., 2005). The correspondence of LAGs to seasonal cycles has been verified in many studies (reviewed in Sinsch, 2015).

In the present study, we use skeletochronology to estimate several life history traits of the Iberian spadefoot toad (*Pelobates cultripes*) from a semiarid zone of the Ebro Valley in the northeastern Iberian Peninsula. *Pelobates cultripes* occurs in the mesomediterranean bioclimatic region of the Iberian Peninsula and in the French Atlantic and Mediterranean coast (Lizana, 1997; Barbadillo et al., 1999; Recuero, 2010). Due to the isolation of populations, agricultural intensification and invasive predators, *P. cultripes* is designated in the IUCN Red List as Near Threatened. Although the phenology and larval period in *P. cultripes* have been relatively well studied (e.g. Álvarez et al., 1990; Lizana et al., 1994; Marangoni & Tejedo, 2008), there are few studies on the biology and ecology of the adults (Cei & Crespo, 1971; Lizana et al., 1994; García-París et al., 2004; Leclair et al., 2005; Marangoni, 2006; Masó & Pijoan, 2011). Specifically, growth and age structure of *P. cultripes* have only been studied in populations in the southwestern Iberian Peninsula (Leclair et al., 2005; Marangoni, 2006; but see also Talavera, 1990; Díaz-Paniagua et al., 2005). The aim of the present study is to analyse the age structure and body size variation in a population of *P. cultripes* from the northeastern Iberian Peninsula, contributing to a better understanding of geographic variation in demographic traits.

MATERIAL AND METHODS

Study area

The study site (240 m a.s.l.) is located in the Mas de Melons area in the valley of the Ebro river (Lleida, Spain). Climate is Mediterranean and moderately continental

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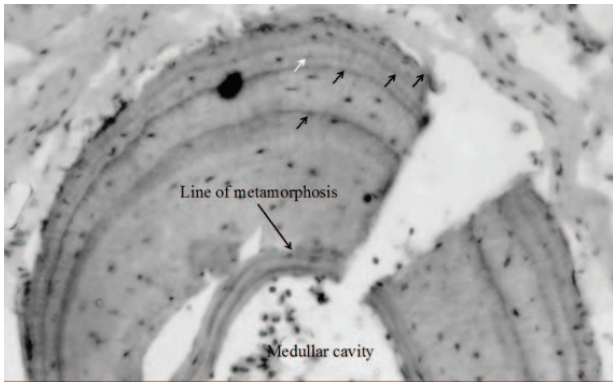

Fig.1. Stained cross-section of a phalange of a *Pelobates cultripes* of 4 years. Black arrow: annual lines, white arrow: non-annual lines.

with average summer temperatures of 24.7°C (with the maximum in July) and winter temperatures of 5.3°C (with the minimum in January). Climate data (1971–2000) were collected from a meteorological station of the Instituto Nacional de Meteorología in Lleida (219.2 m.a.s.l.). The area is considered semiarid with an average annual precipitation of 370 mm, including pasture areas and arable land (winter cereals). Spadefoot toads reproduce in temporary wetlands during the rainy period. For further details of the study site see Sinsch et al. (2007) and Oromi et al. (2010).

Sampling procedure and skeletochronological analysis

A total of 53 adult (27 males and 26 females) and 28 metamorphs of *P. cultripes* were collected during the reproductive period (March–May 2013). Toads were caught by hand and released at the same point of capture after measurement of the snout-vent length (SVL in mm) and weight (g). Adult males were identified by the presence of a glandular pad on the dorsal surface of the arm. The third toe of the right hind limb was clipped at the level of the third phalange of adult sized individuals, and stored in 70% ethanol at room temperature. Bones were decalcified in 3% nitric acid for 6 h, rinsed in water for 1 h and placed in phosphate-buffered saline (PBS) and sucrose 30% for 48 h at 4°C. The toes were cross-sectioned at 16 µm using a freezing microtome and stained with Herlich's hematoxylin for 30 min (Oromi et al., 2012). The sections were observed using a light microscope at 200x and 400x magnifications. In order to estimate the age of

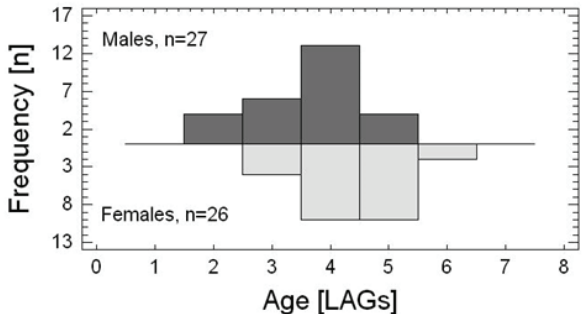

Fig. 2. Age distribution of reproductive males and females of *Pelobates cultripes* population from Mas de Melons.

the adult individuals, LAGs were counted independently by two persons (NO and AM) in the diaphysis bone section in which the size of the medullar cavity was minimum (Fig. 1). The following life history traits were estimated for each sex: age at maturity (minimum age recorded in the reproductive individuals), median age (median of the age distribution), maximum longevity (maximum age recorded) and potential reproductive life span (PRLS, difference between longevity and age at maturity).

Data analysis

All variables were first tested for normality. As age and SVL distributions were significantly skewed, data were normalised by log(10)-transformation. An ANCOVA model was used to analyse the influence of sex and age (considering log-normalised age as a covariate) on log-normalised SVL variation. Differences between the age distributions and median ages of the two sexes were estimated using a Kolmogorov-Smirnov and a Mann-Whitney *U* test, respectively. All analyses were implemented using the STATGRAPHIC 5 package, assuming alpha=0.05. We fitted a Von Bertalanffy growth curve separately for males and females using non linear least-squares in the function *vbfr* from the package *fishmethods* (Nelson, 2014) in R (R Core Team, 2013). We consider the mean size of the 28 metamorphs as starting values of SVL (age=0). Thus, for each sex we obtained the parameters *Linf* (the asymptotic mean size for the population) and *K* (which describes how quickly the asymptote is reached) for the best model in each case (best parameter combination). Once obtained, this parameters are used for the growth equation $Lt=Linf*(1 - e^{(-K*(x-t0))})$. The suitability of these models was

Table 1. ANCOVA for size as log10-normalised SVL.

Source	Sum of squares	Df	Mean square	F-ratio	P-value
Covariate:					
log10(age)	629.07	1	629.07	34.02	<0.0001
Factor					
sex	215.38	1	215.38	10.59	0.002
Residual	1017.15	50	20.34		
Total (corrected)	2441.03	52			

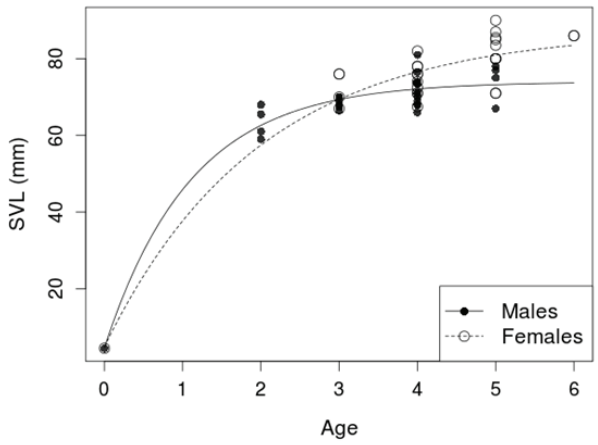

Fig. 3. Von Bertalanffy growth curves comparing males and females of *Pelobates cultripes***.**

evaluated through residual analyses. The significance of the differences between the parameters for males and female models was estimated through likelihood ratio tests using the function *vblrt* in *fishmethods* (Nelson, 2014).

RESULTS

Skeletochronology and age structure

Lines of arrested growth (LAGs) were visible in all cross sections of adult phalanges without the presence of resorption lines. The line of metamorphosis was visible in 90% of individuals (Fig. 1). In the remainder of inddividuals, periosteal bone growth before deposition of the first visible LAG was assumed to correspond to the first-year activity period in which the bone growth rate was at its maximum (following Sinsch et al., 2010). Six individuals showed double lines which could be clearly distinguished from annual marks through weaker staining (see Fig. 1).

The earliest age at sexual maturity was 2 years for males and 3 years for females. The age distributions significantly differed between males and females (Kolmogorov-Smirnov test *DN*=0.70, $p<0.0001$), with a median age of 4 and 5 years for males and females, respectively (Mann Whitney U test, *Z*=497, *p*=0.0061, Fig. 2). Maximum longevity differed between sexes (5 and 6 years for males and females, respectively), resulting in

Table 2. Life history traits of *P. cultripes* (M=males; F=female; AM=age at maturity; MA=median age; Long=longevity; PRLS=potential reproductive life span; mSVL= mean snout-vent length; LAT=latitude; LONG=longitude; ALT=altitude). [a]This study; [b]Talavera, 1990; [c]Leclair et al., 2005; [d]Marangoni, 2006; [e]Díaz-Paniagua, 2005.

Locality	Sex	n	AM [year]	MA [year]	Long [year]	PRLS [year]	mSVL [mm]	LAT	LONG	ALT [m]
Mas de Melons[a]	M	27	2	4	5	3	71.76	41°32’	-0°40’	240
	F	26	3	4	6	3	76.14			
Madrid[b]	M	32	2	7.7	12	10	79.8	40°20’	-3°34’	904
	F	32	2	6.8	12	10	79.5			
Serra da Arriça[c]	M	20	2	4.5	8	6	52.8	38°44’	-8°29’	300
	F	40	2	5	7	5	54.2			
Porto Covo[b]	M	11	2	4.6	7	5	71.5	37°50’	-8°46’	100
	F	4	2	4.5	5	3	72.7			
Aznalcóllar[d]	M	14	2	4.5	8	6	88.1	37°31’	-6°16’	130
	F	7	1	4.4	9	8	104.7			
Bodegones[d]	M	29	2	5	9	7	71.62	37°10’	-6°39’	32
	F	22	1	4.8	11	10	76.2			
Abalario[d]	M	27	2	6.8	11	9	67.67	37°07’	-6°39’	63
	F	29	2	6	10	8	71.84			
RB Doñana[d]	M	24	2	5.2	9	7	66	37°00’	-6°30’	25.5
	F	2	4	6	8	4	62.9			
Doñana[e]	M	8	3	4.9	8	8	55.9	37°00’	-6°30’	25.5
	F	8	3	4.9	8	8	60.8			

a potential reproductive life span (PRLS) of 3 years for both sexes.

Age-size relationship

The log-normalised SVL distribution of individuals differed significantly between males (mean±SE=71.76±0.90 mm) and females (76.14±0.95 mm), and the log-normalised age (covariate) significantly influenced SVL (ANCOVA, table 1). Size at maturity was 63.4±1.9 mm and 72.2±2.5 mm for males and females, respectively. Von Bertalanffy growth curves reveal that *K* values and asymptotic SVL differed between the sexes (Fig. 3). Males grow faster than females until sexual maturity at three years of age, and growing slow than females at reproductive life stages. The asymptote of female SVL (*Linf*=87.47) was significantly larger than that of males (*Linf*=73.96; $\chi^{2=}$15.6, p<0.001). *K* of males (0.904) was significantly larger than in females (0.506; χ^2=10.28, P<0.001). The von Bertalanffy equations are Lt=87.47 * (1-$e^{(-0.506*(t + 0.1139))}$) for females and Lt=73.96 * (1-$e^{(-0.904*(t + 0.0715))}$) for males ($\chi^2$=16.8, p<0.001).

DISCUSSION

The histological interpretation of the LAG formation observed in the diaphysis section in our study conforms to a typical growth pattern for anuran amphibians inhabiting temperate zones (Sinsch, 2015). LAG formation is clearly due to the interruption of growth in the overwintering period, whereas in a population in the south-central Portugal the activity period occurs in winter with an aestivation period marked in the bone LAG formation (Leclair et al., 2005). We however also found six individuals with multiple LAGs, which most probably represent histological equivalents of interrupted growth during periods of drought and elevated ambient temperature (Sinsch et al., 2007). The rapid growth in the first year after metamorphosis of *P. cultripes* is similar to observations made for *Bufo calamita* in the same semiarid zone (Sinsch et al., 2007; Sinsch et al., 2010). Adult size may largely depend on the size achieved at first hibernation or aestivation (Sinsch et al., 2010). First breeders showed large sizes (>70 mm attained at 2 years in males and 3 years in females) probably related to the duration of, and temperature during the activity period to determine food availability (Sinsch et al., 2010). *Pelobates cultripes* from Porto Covo reached 60 mm at 2 years of age (Talavera, 1990), whereas under harsher summer conditions in Serra da Arriça the growth rate is reduced (40 mm SVL when reaching sexual maturity; Leclair et al., 2005).

Similar to most other anurans, we found female *P. cultripes* being larger than males (Shine, 1979). Variation in size dimorphism has been proposed to result from differences in age structure between sexes (Monnet et al., 2002). In our study population, females are both larger and older than males, which grow faster prior to sexual maturity. This result suggests that sexual size dimorphism in *P. cultripes* is largely a consequence of delayed maturity in females, at however comparable reproductive life spans between the sexes due to earlier maturity of males.

In amphibians, age at maturity and longevity usually increase with latitude and altitude (Morrison and Hero, 2003). The demographic studies on *P. cultripes* are summarized in the Table 2, and do not appear to correlate with latitude or altitude, suggesting other proximate mechanisms that determine age and size. The clinal decreases in body size of *P. cultripes* found in Sierra Morena-Doñana area (Marangoni, 2006) appears to be cause by local factors such as food availability and temperature (see Jorgensen, 1992). Although a decrease in size with increasing altitude or latitude is a common feature in anuran populations (e.g. Leskovar et al., 2006; Sinsch, 2015), there is considerable size variation among lowland populations of *P. cultripes* (see Table 2). The reduced size and longevity in toads from Arriça and Doñana are linked to harsh summer conditions which might limit surface activity and reduce survivorship at older age, favouring early maturity at expenses of a larger size (Díaz-Paniagua et al., 2005; Leclair et al., 2005; Marangoni, 2006). This contrasts the Aznalcollar population, where environmental determinants other than altitude or latitude appear to underlie the large sizes in both sexes (Marangoni, 2006).

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