



## CELLULAR AND MOLECULAR BIOLOGY

# Can age and growth patterns explain the geographical variation in the body size of two toad species?

FEDERICO MARANGONI, MIGUEL TEJEDO & DAN COGĂLNICEANU

**Abstract:** Determining both the age structure and growth pattern allows to establish the causal factors, environmental and/or genetic, that eventually may be responsible for the observed pattern of divergence. We examined the variation in age structure and growth pattern across populations of two toad species, *Pelobates cultripipes* and *Epidalea calamita* that exhibit a geographic variation in body size in southern Spain. For both species, populations differed in mean age but age structure did not correlate with body size variation across populations. Although the population with the youngest females found for *E. calamita* was the smallest in body size, the oldest males for both species were found in a small body size population. The growth pattern fit well to a von Bertalanffy growth model and interdemographic divergence were found for both the asymptotic body size ( $S_m$ ) and the growth coefficients ( $k$ ). As expected, Large-Bodied populations of both species attained higher  $S_m$  but, Small-Bodied population had higher, although non significantly different,  $k$  growth coefficients. Also, the Small-Bodied population attained sexual maturity sooner but had also high longevity. The observed pattern may reflect both environmental variations in resources availability affecting body size observed across populations, but also different growth and maturity pathways that may respond to contrasting selective pressures.

**Key words:** Age, Anura, body size, *Epidalea calamita*, growth, *Pelobates cultripipes*.

## INTRODUCTION

Body size is a complex attribute and many factors are responsible for its variation (Angilletta & Dunham 2003). Environmental conditions are determinant of ectotherms body size variation and current knowledge considers temperature as a main driver of large-scale size clines. There is a widespread plastic response of body size to rearing temperatures, with larger individuals occurring at lower temperatures and smaller individuals at warmer temperatures (temperature-size rule hypothesis, Atkinson 1994). For endotherms, Bergmann's rule (Bergmann 1847), states that body size increases

with latitude or altitude. Ray (1960) and Lindsey (1966) suggested that ectothermic species also follow this rule and display intra- and inter-specific body size variation as a response to environmental gradients. While the validity of Bergmann's rule in endothermic species is well-documented (e.g. Ashton 2002a, Freckleton et al. 2003), in ectothermic vertebrates, such as amphibians, conflicting evidences were found for both Bergmann and converse Bergmann clines, reflecting inconsistent large scale patterns across taxa (e.g. Adams & Church 2008, Ashton 2002b, Cvetkovic et al. 2009, Sinsch et al. 2010). In addition, amphibians also may exhibit striking body size variation at smaller

spatial scale, for nearby locations with similar climate characteristics, but with variation in microhabitat conditions, such as substrate nature, that may impose nutritional and/or hydric constraints (Marangoni et al. 2008, Leclair et al. 2005). Body size also varies according to sex: sexual size dimorphism is a population property that describes the intraspecific differences in overall size and shape traits between males and females (Fairbairn 2007). The evolution of sexual size dimorphism is an indirect consequence of the different reproductive roles of males and females (Andersson 1994), emerging from sexual differences in life history (Halliday & Verrell 1988, Halliday & Tejedo 1995, Monnet & Cherry 2002).

Amphibians appear to adapt to temporally uncertain environments with life-history trade-offs, such as variable age at maturity (Wilbur & Rudolf 2006), a strategy called “bet hedging” by Slatkin (1974). Differences in age-related parameters (e.g. age at maturity and longevity) could explain population divergence if individuals from small-bodied populations mature earlier or have a lower survival rate (i.e. reduced longevity), compared to large-bodied ones (e.g. Bruce & Hairston 1990, Cogălniceanu & Miaud 2003). For example, reduced life-span was suggested as the main factor of body size reduction of *Pelobates cultripipes* inhabiting sandy substrates of Sierra de Ariça (Portugal) (Leclair et al. 2005). A significant reduction in body size was reported in both *P. cultripipes* and *Epidalea calamita* populations in southwestern Spain, over a mere 60 km transect associated to a geological transition from Hercinic to sandy substrates. Such reduction in body size was accompanied by a drastic change in clutch mass, clutch size, and egg size in both species (Marangoni et al. 2008). One potential explanation is that body size reduction in these populations is the result of adaptive covariation

among several life-history traits aimed at optimizing fecundity (Sinsch et al. 2010) or simply the result of phenotypic plasticity induced by changes in any environmental factors (e.g. substrate) affecting growth rates (van Kleunen & Fischer 2005). For example, in ectotherms, sexual maturity is delayed and occurs at smaller size when growth rate is lowered by low food availability, while it occurs also later but at larger sizes when growth rate is lowered by decreased temperature (Berrigan & Charnov 1994).

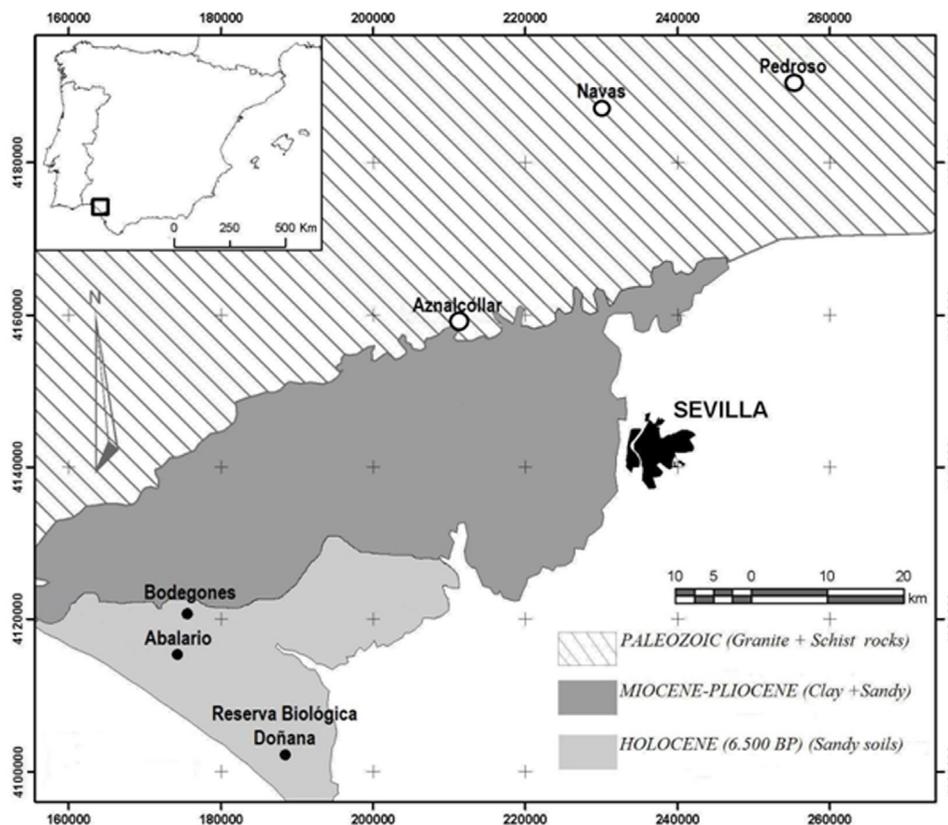
Extreme body size variation in four amphibian species was reported from the sandy substrates from Doñana area, southwestern Spain. Populations of the Southern Marbled Newt (*Triturus pygmaeus*) and Bosca's Newt (*Lissotriton boscai*) have a decrease in snout-vent length (SVL) of 29.0 % in males and 32.2 % in females for *T. pygmaeus* (García-París et al. 1993, Díaz-Paniagua et al. 1996, Díaz-Paniagua & Mateo 1999). Populations of two toad species, the Iberian Spadefoot Toad (*Pelobates cultripipes*) and the Natterjack Toad (*Epidalea calamita*) (Marangoni & Tejedo 2008, Marangoni et al. 2008), exhibit a decrease in body mass of 71.6% and 76.1% and in SVL of 36.8% and 35.6% for *P. cultripipes* and *E. calamita*, respectively, in nearby populations (less than 30 km) inhabiting Hercinic substrates (Marangoni & Tejedo 2008, Marangoni et al. 2008). This variation in body size over a small spatial scale appears to be coupled with changes in age-related parameters, with earlier age of maturity and shorter longevities in dwarf populations of the newt *T. pygmaeus* (Díaz-Paniagua et al. 1996). This suggests an important aspect when establishing geographical variation in amphibian body size; because their indeterminate pattern of growth, implying that body-size continues to increase throughout life, even after reaching sexual maturity, determine the attainment of a larger asymptotic body size (Stamps 1993, Halliday & Tejedo 1995). In

addition, as in most ectotherms, clutch and, sometimes, egg size are positively correlated with amphibian female size (Duellman & Trueb 1986, Wells 2010). Analyzing clinal variation in size and the environmental determinants of intraspecific body size variation requires a parallel knowledge of the relationships between populational variation in age and their correspondence with size and related traits, such as reproductive outputs. In this study, we tested whether interpopulational divergence in age structure and growth are responsible for the variation in body size and reproductive output in two toad species, the Iberian Spadefoot Toad (*Pelobates cultripes*) and the Natterjack Toad (*Epidalea calamita*) along a 120 km transect in southern Spain.

## MATERIALS AND METHODS

### Area and species studied

Based on the previous studies done in the region (Marangoni & Tejedo 2008, Marangoni et al. 2008), we selected six populations for each species at locations distributed along 120 km stretch encompassing two areas with contrasting geological substrates: old Hercinic granite schist substrates of the Sierra Morena (Sevilla province) and Sandy substrates from Quaternary aeolian deposits in the Doñana region (Huelva province) (Fig. 1). The three populations from Hercinic substrate were categorized as Large-Bodied populations (hereafter): Pedroso, Navas and Aznalcóllar, with a range of altitudes between 100 and 420 m. Similarly, the three populations from the Sandy substrates were categorized as Small-Bodied population (hereafter): Bodegones, Abalarío and Reserva Biológica de



**Figure 1.** Location and geological substrate of the studied *Epidalea calamita* and *Pelobates cultripes* populations. Large-Bodied population (open circle) and Small-Bodied population (solid circle). Abbreviated names of sampling populations, geographic coordinates (Coordinates UTM x/y in meters, Datum European 1950, Spain and Portugal, Zone: 30) and elevation, are as follows: PED (255170/4190574, 395 m); NAV (229255/4187617, 420 m); AZN (210199/4158023, 130 m); BOD (175577/4120711, 32 m); ABA (174267/4115417, 63 m); RBD (188450/4102197, 24 m).

Doñana (RBD) with a range of altitudes between 20 and 63 m (Fig. 1). Two out of six *E. calamita* populations (Navas and RBD) were included in a previous study by Sinsch et al. (2010). All studied populations of both species breed in small and shallow temporary ponds that flooded by rainfalls and dry up in the summer and their breeding is synchronic (Marangoni & Tejedo 2008, Marangoni et al. 2008). The main climatic variables do not vary significantly along the transect: annual average rainfall was 640 mm and 585 mm, while average monthly temperature was 25.7°C and 24.5°C in July, and 9.6°C and 10.6°C in January, for Navas and RBD populations, respectively (Díaz-Paniagua 1986, F. Marangoni & M. Tejedo, unpublished data).

### Assessing age-related parameters

We collected 248 *Epidalea calamita* and 149 *Pelobates cultripes* adults from their breeding ponds from fall 1999 to winter 2004. Toad collection methods and body size measurements are detailed in Marangoni et al. (2008). Briefly, calling males or pairs in amplexus were collected during chorusing nights, to ensure that all individuals were mature. Upon capture animals were measured and weighted, and a toe was cut and stored in 70% alcohol for age assessment. All individuals were released back into their original ponds within 24-48 h after their capture. The protocol used for skeletochronology followed the method proposed by Castanet & Smirina (1990) with small modifications (Stănescu et al. 2016). Two independent observers recorded the presence/absence of the line of metamorphosis (LM) and counted the lines of arrested growth (LAG). In those individuals with no remnant LM, we estimated the degree of resorption by osteometrical analysis following Sagor et al. (1998), so that endosteal resorption did not prevent age estimation. We distinguished annual growth marks (i.e., LAGs *sensu stricto*) from

non-annual ones (i.e., irregular interruptions during periods of inactivity), using the method described in Sinsch et al. (2007). Annuli (*sensu* Peabody 1958) were easily distinguishable from actual LAGs since they always stained weaker than true LAGs and were often broader, as previously described by Leclair et al. (2005) and Sinsch et al. (2007) in temperate species. Occasionally, in some individuals, one or two annuli were visible within the summer growth period. We assumed that the number of LAGs is equivalent to the number of aestivations experienced by each individual, thus giving a direct estimation of individual age.

### Demographic life history traits

For each reproductive individual analyzed we obtained the following five, sex-specific and age-related, variables (*sensu* Leskovar et al. 2006): (1) age at sexual maturity: the minimum number of LAGs; (2) longevity: the maximum number of LAGs; (3) potential reproductive lifespan: the difference between longevity and age at sexual maturity; (4) mean lifespan: mean of age distribution; and, (5) size at sexual maturity: the average snout-vent length of all first breeders with the minimum number of LAGs.

### Growth Estimation

We estimated the average body growth of toads (SVL) using von Bertalanffy growth equation (von Bertalanffy 1938):

$$S_t = S_m - (S_m - S_0) e^{-k(t-t_0)}$$

where  $t$  = number of growing seasons experienced (age);  $t_0$  = age at metamorphosis (proportion of the growing season already elapsed at metamorphosis);  $S_t$  = average body size after having experienced  $t$  growing seasons;  $S_m$  = average maximal body size;  $S_0$  = average body size at metamorphosis;  $k$  = growth coefficient, defining the slope of the

growth curve, measuring the exponential rate of approach to  $S_m$ . Fair estimates of  $S_0$  for each population, and thus allowing a better model adjustment, were obtained as average body size at metamorphosis from two independent larval growth experiments (*P. cultripipes*: Marangoni & Tejedo 2008, *E. calamita*: Marangoni 2006, see Table IV). Additionally, for fitting *P. cultripipes* growth models, we used mean body size of 5-month-old juveniles, obtained from another study (Marangoni 2006).

### Reproductive effort

We used relative clutch mass (RCM) as an estimate of reproductive effort, calculated as the ratio of clutch mass to spent body mass (Shine 1992, Bonnet et al. 2003). Reproductive traits of the amplexant pairs such as clutch and egg size were obtained following Marangoni et al. (2008). Briefly, the amplexant pairs were assigned to plastic containers where they quickly went back into amplexus and oviposition took place within 12 h. The resulting egg clutches were photographed and the eggs counted and measured. Clutch mass was obtained by subtracting the female body mass before and after deposition. All individuals were released back into their original ponds within 24-48 h after their capture.

### Statistical analyses

We computed a sexual dimorphism index (SDI) for SVL, body mass and average age, with the results arbitrarily defined as positive when females are larger than males and negative in the converse situation (Lovich & Gibbons 1992), since it allows to compare all three parameters (but see Ranta et al. 1994):

$$SDI = \frac{\text{Mean size}_{\text{larger sex}}}{\text{Mean size}_{\text{smaller sex}}}$$

Where “Mean size” refers to either SVL, body mass or average age. The value of SDI is equal (if there is not sexual dimorphism) or greater than unit (when one of the sexes has a larger mean size). The results are defined arbitrarily as positive when females are larger than males and negative when males are larger.

All variables were log-transformed to achieve normality. Two-tailed *t*-test compared mean age between sexes and species and the Kolmogorov–Smirnov tested populational variation in age distribution frequencies. Univariate analyses of variance (ANOVA) tests for differences in mean age and RCM between the sexes, environments (Hercinic versus Sandy substrates) and populations, within environments. Population was considered a random effect throughout the analyses. We adjusted *P*-values by the sequential Bonferroni procedure, when multiple comparisons or tests were performed (Rice 1989). The von Bertalanffy growth model was fitted employing the least square procedure. We used type III general linear models using the STATISTICA 6.0 statistical package (StatSoft Inc. 2001). Mean values were compared using post-hoc Scheffé multiple comparison test, at  $\alpha = 0.05$ . We used linear regression to test the association between age and body size. All results are expressed as mean  $\pm$  1 standard error (SE).

## RESULTS

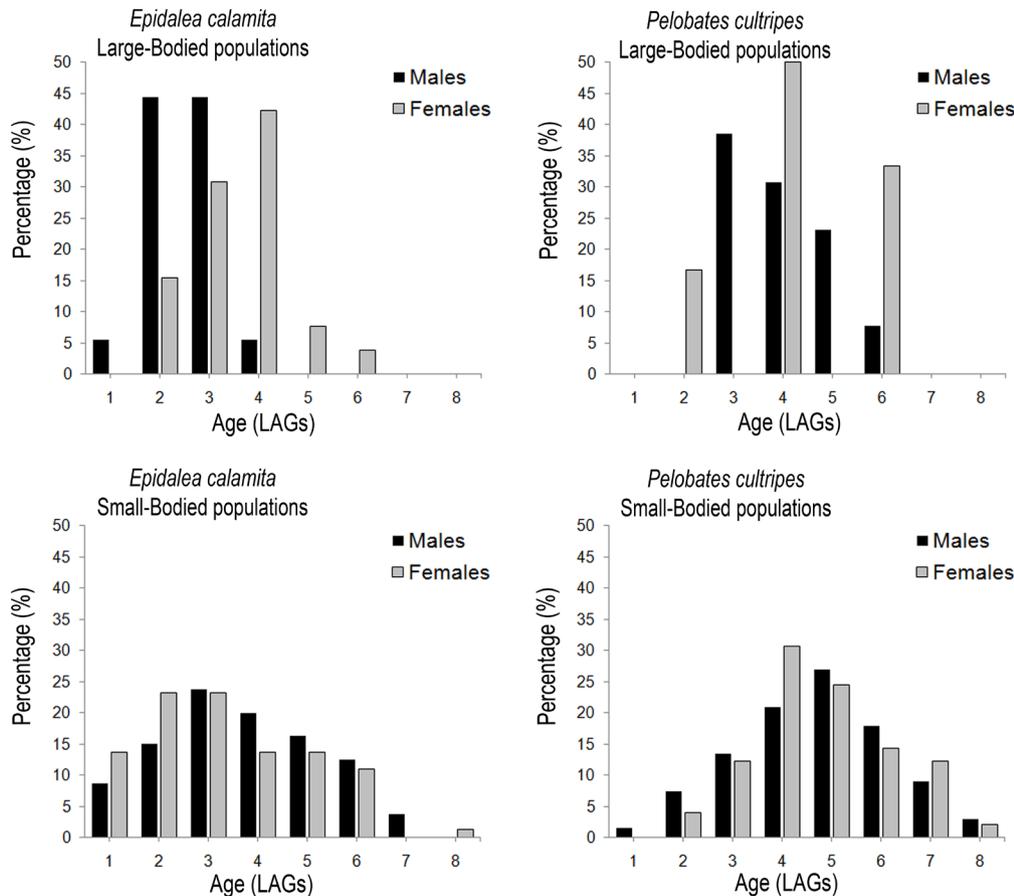
Lines of Arrested Growth (LAGs) were well defined in the periosteal bone and allowed a proper age estimation in all individuals of both species. For those specimens in which resorption occurred during the formation of endosteal bone, at least 76% and 85% of the first LAG (*E. calamita* and *P. cultripipes*, respectively) the LM was never completely eroded. The age

**Table I.** Age and size-related parameters of Small (Sandy substrate) and Large-Bodied populations (Hercinic substrate) of *Epidalea calamita* and *Pelobates cultripes*. AM = age at sexual maturity of the youngest first breeders; PRLS = potential reproductive lifespan, computed as the difference between longevity and AM; SVL = snout-vent length (mm), BM: body mass (g). SDI: sexual dimorphism index. All values for Age, SVL, BM and Minimum SVL at AM are expressed as mean ± 1 SE, [n]: sample size.

Species / Populations	Sex	Age (LAGs)	AM (LAGs)	Longevity (LAGs)	RCM	PRLS (yr)	SVL (mm)	BM (g)	Minimum SVL at AM	Maximum SVL (age)
<b><i>E. calamita</i></b>										
Large-Bodied	♂	2.46 ± 0.11 [39]	1	4		3	72.64 ± 0.53 [177]	32.61 ± 0.92 [115]	61.5 ± 4.5 [2]	88(3)
	♀	3.6 ± 0.17 [43]	2	8	0.41 ± 0.02 (77)	6	71.43 ± 0.71 [98]	28.5 ± 0.92 [77]	67.4 ± 3.75 [5]	85(5)
	♂+♀	2.94 ± 0.12 [82]	1	8		7	72.24 ± 0.43 [275]	31.04 ± 0.68 [192]	---	---
	SDI	+1.46					-1.01	-1.14	+1.09	-1.04
Small-Bodied	♂	3.79 ± 0.18 [89]	1	7		6	55.04 ± 0.60 [147]	12.79 ± 0.42 [147]	51.89 ± 1.34 [9]	77(4)
	♀	3.27 ± 0.19 [77]	1	8	0.47 ± 0.02 (102)	7	53.53 ± 0.65 [105]	11.09 ± 0.44 [105]	50.55 ± 2.92 [11]	86(6)
	♂+♀	3.52 ± 0.13 [166]	1	8		7	54.41 ± 0.44 [252]	12.08 ± 0.31 [252]	---	---
	SDI	-1.16					-1.03	-1.15	-1.03	+1.12
<b><i>P. cultripes</i></b>										
Large-Bodied	♂	4.16 ± 0.34 [19]	2	8		6	85.92 ± 0.76 [101]	45.46 ± 1.28 [101]	75[1]	99(5)
	♀	4.64 ± 0.41 [11]	2	6	0.35 ± 0.13 (4)	4	96.02 ± 2.03 [37]	60.14 ± 5.67 [27]	85[1]	125(6)
	♂+♀	4.11 ± 0.28 [30]	2	8		6	88.63 ± 0.86 [138]	48.56 ± 1.64 [128]	---	---
	SDI	+1.12					+1.12	+1.32	+1.13	+1.26
Small-Bodied	♂	4.67 ± 0.18 [72]	1	8		7	67.30 ± 0.58 [191]	22.0 ± 0.52 [188]	57[1]	86(6)
	♀	4.83 ± 0.2 [47]	2	8	0.29 ± 0.02(64)	6	71.52 ± 0.98 [134]	23.16 ± 0.89 [112]	79 ± 1 [2]	97(6)
	♂+♀	4.72 ± 0.14 [119]	1	8		7	68.85 ± 0.55 [325]	22.33 ± 0.47 [300]	---	---
	SDI	+1.03					+1.06	+1.05	+1.39	+1.13

structure and age-size related parameters are presented in Table I and Fig. 2. The differences between frequency distributions of age between substrates (Hercinic vs. Sandy) by sex and, between sexes within substrate are presented, in Table II. Body size differed between populations occurring at different soil substrates, populations with smaller individuals of both species were found in the Sandy substrates populations (E. calamita, SVL:  $F_{1,213} = 128.99, P < 0.0001$ , BM:  $F_{1,213} = 211.59, P < 0.0001$ ; P. cultripes: SVL:  $F_{1,112} = 124.73, P < 0.0001$ , BM:  $F_{1,112} = 175.72, P < 0.0001$ , Table I). The sexual dimorphism index (SDI) showed differences in body size between the sexes in P. cultripes, with females larger than males, whereas in E. calamita there are no differences in SVL between sexes, but males have higher body mass compared to females

(Table I; see also Table I in Marangoni et al. 2008). Age class distributions between Large and Small-Bodied populations varied only in E. calamita males, but not in E. calamita females and both sexes in P. cultripes (Table II). As a consequence, mean age showed a significant “sex x substrate” interactions in E. calamita (Table III, Fig. 4). E. calamita males from Small-Bodied populations were on average older than Large-Bodied ones, but females from different sized populations were identical in mean age (Figure 4, Table I and Table III). Additionally, this “sex x substrate” interaction indicates that females were significantly older than males in Large-Bodied populations, but not mean age differences between sexes were found in Small-Bodied ones (Table I, Fig. 4).



**Figure 2.** Age distribution of Small-Bodied (Upper) and Large-Bodied (Lower) populations of males and females of *Epidalea calamita* and *Pelobates cultripes*.

There was mean age populational variation within particular substrate in both *E. calamita* (Population (substrate):  $P < 0.01$ , Table III, Fig. 3) and *P. cultripes*, (only examined within the Sandy substrates),  $F_{2,110} = 7.521$ ,  $P < 0.001$ ). Both species showed that ABA population has the older mean age differing from all analyzed populations (post hoc Scheffé multiple comparison test, the lowest  $P$  value found, *E. calamita*:  $P < 0.0001$ , *P. cultripes*:  $P = 0.002$ ), (Fig. 3, Appendix A). Finally, no other sources of mean age variation, such as sex or substrate main effects were significant (Table III).

RCM variation was not explained by population origin, substrate / size for both species and only a population effect was found in *P. cultripes* (Table III). Within the Small-Bodied sandy substrate populations, RBD

(the Smallest-Bodied population) showed the highest reproductive effort ( $0.40 \pm 0.02$  (7)), even higher than Large-Bodied population, although it only was significantly different from BOD population (post-hoc comparisons, Scheffé range test, RCM: RBD vs BOD,  $P = 0.0398$ ). Also, despite “population within environment” effects were not observed in *E. calamita*, the RBD (the Smallest-Bodied population) also showed the highest reproductive effort among all populations ( $0.49 \pm 0.03$  (35)).

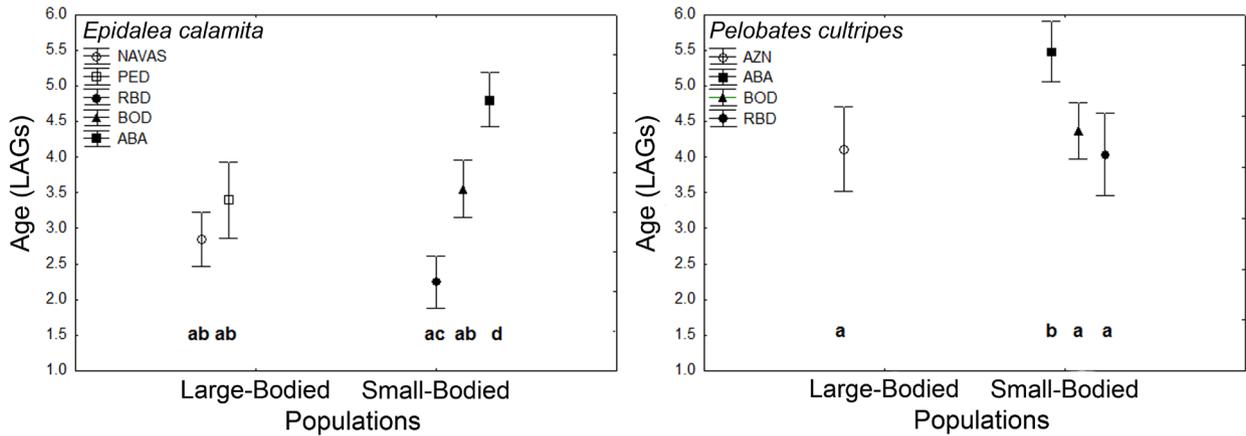
The patterns of growth for body size fits well with a von Bertalanffy’s growth model (Table IV, Fig. 5) and interpopulation divergences were found for both the estimated asymptotic body size ( $S_m$ ) and the growth coefficient ( $k$ ). As expected, Large-Bodied populations of both species attained higher asymptotic size ( $S_m$ ),

**Table II. Comparison of frequency distributions of age between substrates (Hercinic; large-bodied populations; Sandy: Small-bodied populations) by sex (a), and between sexes within substrate.  $n_L$  and  $n_S$  frequency of Large and Small-bodied individuals, respectively (b). Kolmogorov-Smirnov test frequency. Significant values are highlighted in boldface.**

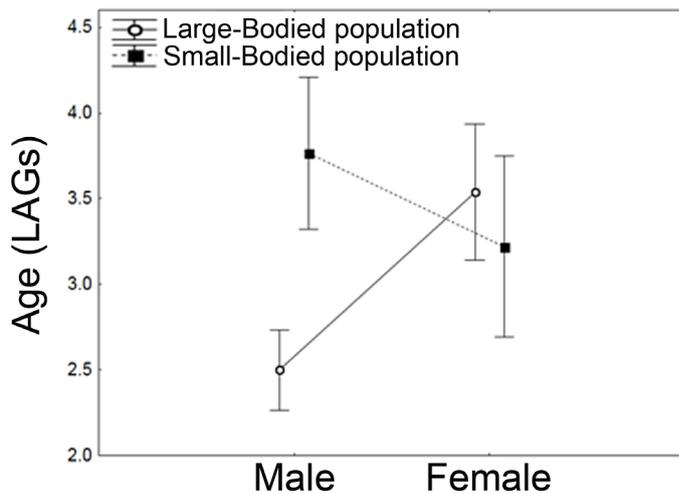
<i>Epidalea calamita</i>	$n_L$	$n_S$	Dmax	P
Sex / Substrate	Large-Bodied vs Small-Bodied			
♂	36	80	-0.469	<b>&lt;0.001</b>
♀	26	73	0.216	>0.10
♂+♀	62	153	-0.246	<b>&lt; 0.01</b>
Substrate / Sex	Males vs Females			
Large-Bodied	36	26	-0.483	<b>&lt;0.005</b>
Small-Bodied	80	73	0.132	>0.10
Large + Small Bodied	124	99	-0.055	>0.10
<i>Pelobates cultripes</i>				
Sex / Substrate	Large-Bodied vs Small-Bodied			
♂	13	67	-0.259	>0.10
♀	5	49	-0.159	>0.10
♂+♀	18	116	-0.218	>0.10
Substrate / Sex	Males vs Females			
Large-Bodied	13	5	-0.323	>0.10
Small-Bodied	67	49	-0.060	>0.10
Large + Small Bodied	80	54	-0.083	>0.10

**Table III.** Results of two-way mixed model nested ANOVAs for responses of *Epidalea calamita* and *Pelobates cultripes* populations to substrate (Hercinic, Large-Bodied population vs Sandy, Small-Bodied populations), accounting for variation among sexes. Response variable are Mean Age and Relative Clutch Mass (RCM). Significant values are highlighted in boldface.

Source	Mean Age						Mean RCM					
	<i>E. calamita</i>			<i>P. cultripes</i>			<i>E. calamita</i>			<i>P. cultripes</i>		
	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P
Substrate	1	0.16	0.719	1	0.04	0.845	1	3.11	0.180	1	0.13	0.727
Population (Substrate)	3	<b>31.34</b>	<b>0.009</b>	4	3.24	0.140	3	1.24	0.297	2	<b>4.19</b>	<b>0.020</b>
Sex	1	2.25	0.217	1	0.01	0.937						
Sex x Population (Substrate)	3	1.49	0.217	4	1.66	0.162						
Sex x Substrate	1	<b>14.54</b>	<b>0.023</b>	1	0.61	0.448						
Error	238			135			174			64		



**Figure 3.** Mean age variations among populations of *Epidalea calamita* and *Pelobates cultripes*. Values for both sexes are pooled. Open = Large-Bodied populations, solid = Small-Bodied populations. All values are means  $\pm$  1 SE. Different letters mean significant differences using a Scheffé post hoc multiple comparison test at  $\alpha = 0.05$ .



**Figure 4.** Effects of sex x substrate interaction on age in *Epidalea calamita*.

**Table IV.** Estimated parameters from von Bertalanffy’s equation, for body growth (SVL) of *Epidalea calamita* and *Pelobates cultripes*.  $S_m$  = average maximal body size,  $S_0$  = body size at metamorphosis (see text for details).  $k$  = growth coefficient, defining the shape of the growth curve,  $r^2$  = model fit. SDI: sexual dimorphism index. All values are means  $\pm$  1 SE (CI 95%).

Species / Populations	Sex	SVL (mm)			
		$S_m$	$S_0$	$k$	$r^2$
<b><i>E. calamita</i></b>					
Large-Bodied	♂	69.94 $\pm$ 1.18 (67.59 – 72.29)	13.28 $\pm$ 0.54 (12.2 – 14.35)	1.51 $\pm$ 0.23 (1.05 – 1.97)	0.988
	♀	74.77 $\pm$ 1.94 (70.92 – 78.62)	13.29 $\pm$ 0.69 (11.90 – 14.67)	0.81 $\pm$ 0.13 (0.56 – 1.06)	0.982
	♂+♀	71.78 $\pm$ 1.10 (69.61 – 73.95)	13.31 $\pm$ 0.74 (11.85 – 14.76)	1.20 $\pm$ 0.15 (0.91 – 1.50)	0.979
	SDI	+1.07		-1.86	
Small-Bodied	♂	59.99 $\pm$ 0.66 (58.72 – 61.27)	12.94 $\pm$ 0.82 (11.31 – 14.57)	1.43 $\pm$ 0.15 (1.13 – 1.72)	0.972
	♀	55.33 $\pm$ 0.82 (53.70 – 56.95)	12.86 $\pm$ 0.99 (10.92 – 14.82)	1.90 $\pm$ 0.31 (1.28 – 2.52)	0.956
	♂+♀	58.05 $\pm$ 0.59 (56.89 – 59.22)	13.01 $\pm$ 1.01 (11.02 – 15.01)	1.53 $\pm$ 0.15 (1.24 – 1.82)	0.940
	SDI	-1.08		+1.33	
<b><i>P. cultripes</i></b>					
Large-Bodied	♂	100.08 $\pm$ 3.64 (92.88 – 107.28)	29.95 $\pm$ 0.57 (28.83 – 31.07)	0.40 $\pm$ 0.05 (0.31 – 0.49)	0.959
	♀	118.00 $\pm$ 8.17 (102.65 – 134.98)	30.49 $\pm$ 0.59 (29.31 – 31.66)	0.26 $\pm$ 0.04 (0.17 – 0.35)	0.950
	♂+♀	101.99 $\pm$ 3.42 (95.23 – 108.75)	29.81 $\pm$ 0.68 (28.47 – 31.16)	0.41 $\pm$ 0.05 (0.32 – 0.50)	0.961
	SDI	+1.17		-1.54	
Small-Bodied	♂	73.05 $\pm$ 0.82 (71.45 – 74.66)	29.70 $\pm$ 0.38 (28.95 – 30.46)	0.63 $\pm$ 0.04 (0.55 – 0.71)	0.967
	♀	79.22 $\pm$ 1.24 (76.78 – 81.67)	29.65 $\pm$ 0.42 (28.81 – 30.48)	0.55 $\pm$ 0.04 (0.46 – 0.63)	0.961
	♂+♀	74.96 $\pm$ 0.81 (73.37 – 76.55)	29.52 $\pm$ 0.47 (28.59 – 30.46)	0.64 $\pm$ 0.05 (0.56 – 0.73)	0.961
	SDI	+1.08		-1.15	

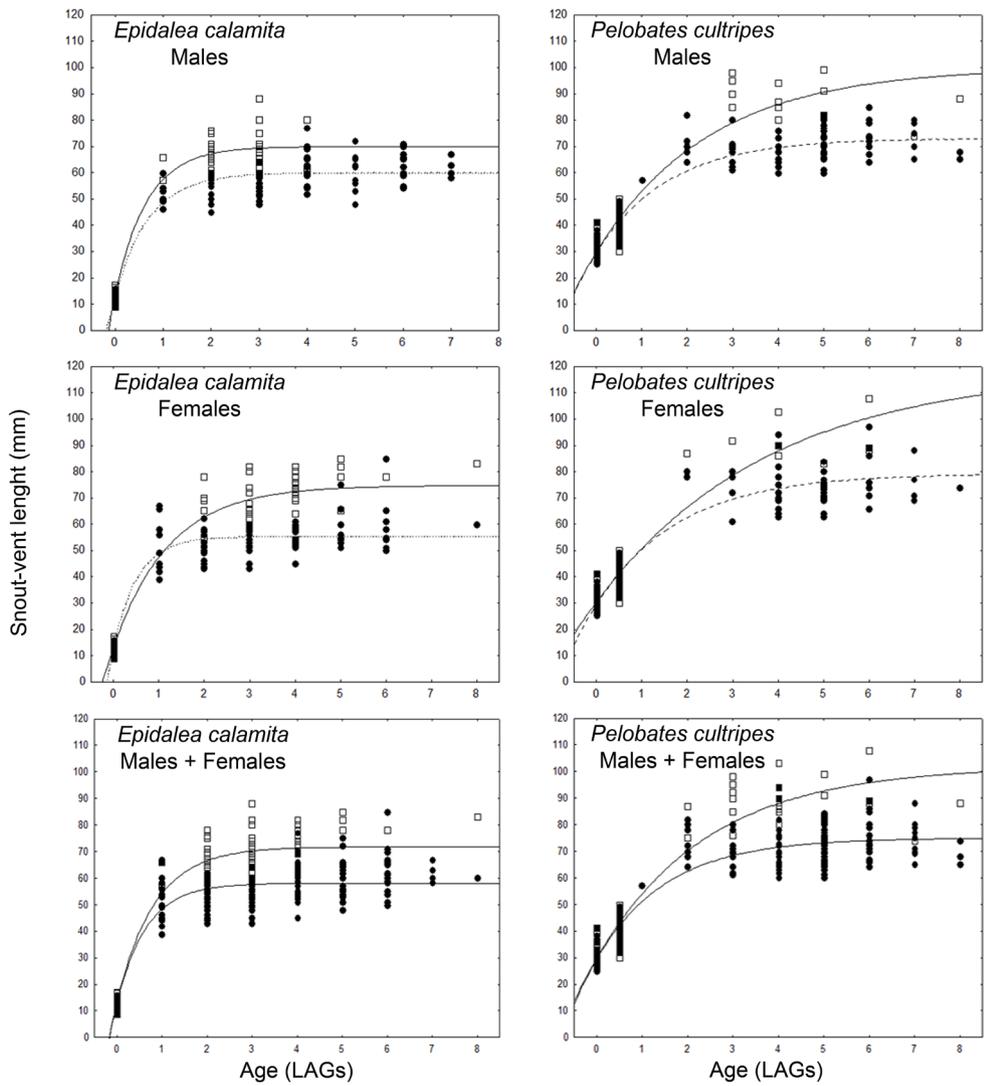
but Small-Bodied population had higher growth coefficients, although the differences are not significant (Table IV and Table V).

### DISCUSSION

Our study showed that interdemographic differences in age and growth are only partially responsible for the extreme reduction in body size in two toad species over a short geographical range associated to soil substrate differences. Thus, the Small-Bodied populations compensate for smaller size by achieving earlier sexual maturity. This allows for a longer potential reproductive lifespan, thus compensating for the lower number of eggs deposited during a single annual

reproductive event (fig. 6). Thus, females from both populations tend towards depositing a similar number of eggs during their life-span by either growing a larger body size (and producing a larger clutch size), or maturing earlier and increasing the reproductive life-span.

Several factors can contribute to small adult sizes in amphibians: reduced egg size, reduced larval and/or juvenile growth and early sexual maturation. Small females often tend to lay smaller eggs (Semlitsch & Gibbons 1990, Michimae 2007, Davenport & Summers 2010, Vignoli et al. 2018) which in turn tend to yield smaller metamorphs (Bernardo 1996). Variation in larval growth may affect adult size, especially when compensatory post-metamorphic growth does not take place (Metcalf & Monaghan



**Figure 5.** Growth curves fitted to the von Bertalanffy model, for body growth (SVL) in *Epidalea calamita* (left panels) and *Pelobates cultripes* (right panels). Large-Bodied population – top-line; Small-Bodied population – bottom line.

2001), but differences in growth rate between juveniles may fill the body size gap (Vignoli et al. 2018, Székely et al. 2020). Post-metamorphic individuals exhibit indeterminate growth that declines when sexual maturity is attained (Hemelaar 1988). Differences in larval growth partially explain the divergence in size at metamorphosis, accounting for 20% of total population divergence in adult size found between the toad populations from different substrates, as indicated by common garden experiments (Marangoni & Tejedo 2008). Our study highlighted that age-related parameters

are partly responsible for this pattern of size variation.

There are differences between sexes in mean age, with males having a lower mean age, except for Small-Bodied *E. calamita* populations, where males live longer. Longevity (i.e. highest age class observed) is higher in females in *E. calamita*. The differences between sexes in mean age are highest in the Large-Bodied populations. The differences in mean age and longevity are smaller in *P. cultripes*, compared with *E. calamita*.

Both species present a steep reduction in body size and body mass, within just about 30

**Table V.** T-student test for differences between Large-Bodied (LBP) and Small-Bodied populations (SBP) in estimated parameters from von Bertalanffy equations, for body growth (SVL) of *Epidalea calamita* and *Pelobates cultripipes*.  $S_m$  = average maximal body size,  $k$  = growth coefficient, defining the slope of the growth curve. Significant values are highlighted in boldface.

Species	Sex	$S_m$ (LBP vs SBP)			$k$ (LBP vs SBP)		
		t	df	P	t	df	P
<i>E. calamita</i>	♂	4.978	114	<b>&lt;0.001</b>	0.086	114	0.466
	♀	7.945	97	<b>&lt;0.001</b>	-0.805	97	0.211
	♂+♀	7.040	213	<b>&lt;0.001</b>	-0.355	213	0.361
<i>P. cultripipes</i>	♂	8.606	78	<b>&lt;0.001</b>	-0.350	78	0.364
	♀	7.610	52	<b>&lt;0.001</b>	-0.326	52	0.373
	♂+♀	7.970	132	<b>&lt;0.001</b>	-0.283	132	0.389

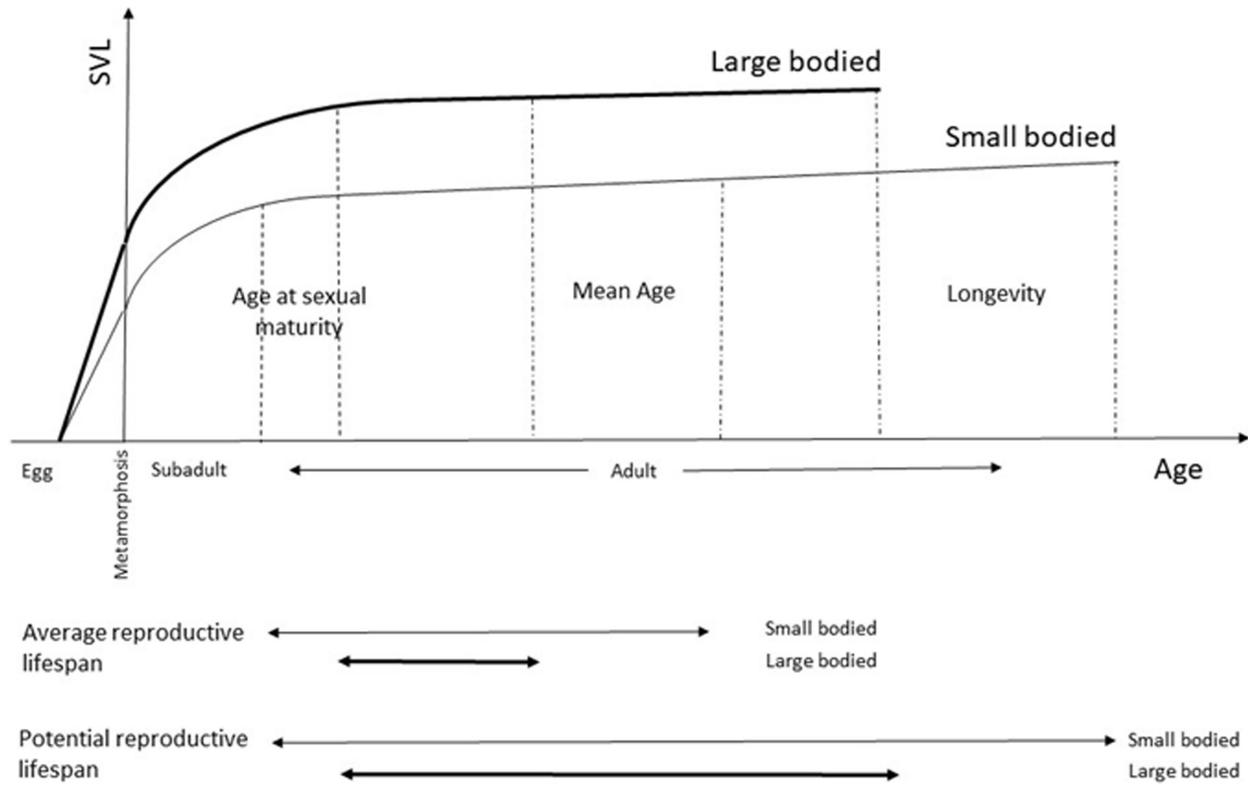
km, in association with the Sandy substrates (Marangoni et al. 2008). Such reduction in body size also causes a parallel decrease in reproductive output and particularly on egg size in both species (Marangoni et al. 2008), and a reduced body size at metamorphosis in both species (Marangoni 2006, Marangoni & Tejedo 2008). Since juveniles from the Small-Bodied populations metamorphose later and at a smaller size compared with those from Large-Bodied populations (Marangoni & Tejedo 2008), they compensate by reaching sexual maturity rapidly, after the first year, and afterwards almost stop growing, as indicated by the higher  $k$  values. Thus, most energy is directed towards reproduction and little to growth in Small-Bodied populations.

Age and body size at sexual maturity indicate how fast individuals can start reproducing and are also an indicator of their reproductive output, since fecundity is often associated with body size (Plaistow et al. 2004). A study of two syntopic species of the genus *Pelobates* that differ in their sexual size dimorphism showed that different growth rates before sexual maturity and differences in energy allocation between growth and reproduction after sexual

maturity are responsible for the differences in adult size (Cogălniceanu et al. 2014).

The reasons and implications of sexual size dimorphism in anurans have been extensively debated (Shine 1979, Woolbright 1983, Halliday & Verrell 1986, Monnet & Cherry 2002), but no clear pattern is apparent. It could be due to male–male interactions (Shine 1979), female choice for larger males (Halliday & Verrell 1988), or differences in age structure between sexes that lead to differences in body size (Miaud et al. 1999, Monnet & Cherry 2002, Liao & Chen 2012). Shine (1979) reviewed for the first time the literature on sexual size dimorphism and observed that in 90% of anurans, females grow larger than males. Sexual size dimorphism is present in *P. cultripipes* with females larger and heavier than males, while in *E. calamita* sexual dimorphism is absent regarding body size, but, rather unusual, males are heavier than females. This suggests that age alone is not responsible for these differences.

Fecundity selection in females and sexual selection in males are considered the major evolutionary forces selecting for larger body size in most organisms (Blanckenhorn 2000). Halliday & Verrell (1988) suggested that female choice of larger males favors males with rapid



**Figure 6.** A schematic comparison of size and age-related parameters in Large and Small-Bodied populations.

juvenile growth, and not just older age. This might explain the rapid growth pattern of males in Large-Bodied populations of both species, as an advantageous trait for reproduction. The positive correlation between female body size and clutch size (Duellman & Trueb 1986) suggests a benefit to male–male competition for larger females. Selection for large body size is eventually counterbalanced by opposing selective forces, primarily viability selection (Andersson 1994, Blanckenhorn 2000). Increased body size will be favored only if it decreases mortality or enhances reproductive success sufficiently to compensate for the increased production required by a larger body mass (Brown & Sibly 2006).

Previous studies that estimated age related parameters through skeletochronology indicate important variation in both size and age among populations (Appendix A). The *P. cultripes*

population from Serra da Arica (Leclair et al. 2005) is even smaller in size than our Small-Bodied populations. Overall, we report here for the first time age at sexual maturity of 1 year, smaller than the reported 2-4 years in other studies on *P. cultripes* or 2-7 in other species of the genus *Pelobates*. Longevity is also lower in the studied populations (8 years old) compared to a maximum of 12 years in a population from Madrid (Talavera 1990). A complete review of age-related studies in *E. calamita* was done by Sinsch (2015). Age at sexual maturity varies greatly and is usually higher than our reported 1 year, reaching up to 5 years old. Longevity varies largely: in three populations it was over 10 years, but in other populations is shorter, of even 4 years, i.e. half of our reported 8 years. Longevity is influenced by sample size, i.e. a larger sample means a higher probability to find

older individuals, so comparisons should be made with caution.

The development and growth, and thus the size of most organisms, are affected by temperature and water availability (Sheridan & Bickford 2011). For ectotherms, metabolic rates directly scale with temperature, thus an increase in metabolism associated with climate warming will reduce the body size of ectotherms unless organisms can compensate with greater food intake or reallocating caloric resources. Increased temperatures can also lead to smaller ectotherms by increasing developmental rate (termed the temperature–size rule). Decreasing body size has been proposed as a universal response to increasing temperatures (Baudron et al. 2014). Body-size reduction is thus the third universal response to global warming, alongside changes in the phenology and distributions of species. Apart from the direct effect of temperature on body size, one other probable proximate cause of changing body size is a change in the availability or quality of food (Gardner et al. 2011). Since in our study air temperature does not differ between large and small-bodied populations, we suggest that differences in food availability or quality caused by substrate are responsible for the observed variation of body size.

Overall, our study identified an interesting pattern in life-history strategies, with populations from two different species having similar trade-offs between size, growth and age, to compensate for environmental variation.

### Acknowledgments

We thank F. Campos and C. Díaz-Paniagua for their invaluable help at laboratory and skeletochronological method, respectively. F. Stănescu reviewed earlier drafts of this manuscript, providing useful comments that greatly improved the paper. Two anonymous reviewers provided constructive comments. We thank H. Duarte, a native speaker, for correcting the English draft of this

manuscript. This work was supported by grant PB96–0861 from Dirección General de Investigación Científica y Técnica conceded to M. Tejedo. Thanks also to the Consejería de Medio Ambiente de la Junta de Andalucía and the Reserva Biológica de Doñana, for providing the corresponding permits and facilities. All animal experimentation was conducted in accordance with the legal standards of Spain.

### REFERENCES

- ADAMS DC & CHURCH JO. 2008. Amphibians do not follow Bergmann's rule. *Evolution* 62: 413–420.
- ANDERSSON M. 1994. *Sexual Selection*. New Jersey, Princeton: Princeton University Press.
- ANDREONE F, EUSEBIO BERGÒ P, BOVERO S & GAZZANIGA E. 2004. On the edge of extinction? The spadefoot *Pelobates fuscus insubricus* in the Po Plain, and a glimpse at its conservation biology. *Ital J Zool* 71: 61–72.
- ANGILLETTA MJ & DUNHAM AE. 2003. The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. *Am Nat* 162: 332–342.
- ASHTON KG. 2002a. Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecol Biogeogr* 11: 505–523.
- ASHTON KG. 2002b. Do amphibians follow Bergmann's rule? *Can J Zool* 80: 708–716.
- ATKINSON D. 1994. Temperature and organism size: a biological law for ectotherms? *Adv Ecol Res* 25: 1–58.
- BAUDRON AR, NEEDLE CL, RIJNSDORP AD & TARA MARSHALL C. 2014. Warming temperatures and smaller body sizes: synchronous changes in growth of North Sea fishes. *Glob Change Biol* 20: 1023–1031.
- BERGMANN C. 1847. Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Göttinger Stud* 1: 595–708.
- BERNARDO J. 1996. The particular maternal effects of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *Am Zool* 36: 216–236.
- BERRIGAN D & CHARNOV EL. 1994. Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. *Oikos* 70: 474–478.
- BLANCKENHORN WU. 2000. The evolution of body size: what keeps organisms small? *Q Rev Biol* 75: 385–407.

- BONNET X, SHINE R, LOURDAIS O & NAULLEAU G. 2003. Measures of reproductive allometry are sensitive to sampling bias. *Funct Ecol* 17: 39-49.
- BROWN JH & SIBLY RM. 2006. Life-history evolution under a production constraint. *P Natl Acad Sci USA* 103: 17595-17599.
- BRUCE RC & HAIRSTON NG. 1990. Life-history correlates of body-size differences between two populations of the salamander, *Desmognathus monticola*. *J Herpetol* 24: 124-134.
- CASTANET J & SMIRINA E. 1990. Introduction to the skeletochronological method in amphibian and reptiles. *Ann Sci Nat Zool Paris* 11: 191-196.
- COGĂLNICEANU D & MIAUD C. 2003. Population, age structure and growth of four amphibian species inhabiting a large river floodplain. *Can J Zool* 81: 1096-1106.
- COGĂLNICEANU D, ROȘIORU D, SZÉKELY P, SZÉKELY D, BUHACIUC E, STĂNESCU F & MIAUD C. 2014. Age and body size in populations of two syntopic spadefoot toads (genus *Pelobates*) at the limit of their ranges. *J Herp* 48: 537-545.
- CVETKOVIC D, TOMASEVIC N, FICETOLA GF, CRNOBRNJA-ISAILOVIC J & MIAUD C. 2009. Bergmann's rule in amphibians: combining demographic and ecological parameters to explain body size variation among populations in the common toad *Bufo bufo*. *J Zool Syst Evol Res* 47: 171-180.
- DAVENPORT JM & SUMMERS K. 2010. Environmental influences on egg and clutch sizes in lentic and lotic breeding salamanders. *Phyllomedusa* 9: 87-98.
- DÍAZ-PANIAGUA C. 1986. Reproductive period of amphibians in the biological reserve of Doñana (SW Spain). In: Roček Z (Ed). *Studies in Herpetology*, Prague: Charles University, p. 429-432.
- DÍAZ-PANIAGUA C & MATEO JA. 1999. Geographic variation in body size and life-history traits in Bosca's newt (*Triturus boscai*). *Herpetol J* 9: 21-27.
- DÍAZ-PANIAGUA C, GÓMEZ-RODRÍGUEZ C, PORTHEAULT A & DE VRIES W. 2005. Los Anfíbios de Doñana. Organismo Autónomo de Parques Nacionales. Ministerio de Medio Ambiente, Madrid.
- DÍAZ-PANIAGUA C, MATEO JA & ANDREU AC. 1996. Age and size structure of populations of small marbled newts (*Triturus marmoratus pygmaeus*) from Doñana National Park (SW Spain). A case of dwarfism among dwarfs. *J Zool* 239: 83-92.
- DUELLMAN WE & TRUEB L. 1986. *Biology of amphibians*. New York: McGraw-Hill.
- EGGERT C & GUYÉTANT R. 1999. Age structure of a spadefoot toad *Pelobates fuscus* (Pelobatidae) population. *Copeia* 1999: 1127-1130.
- EGGERT C & GUYÉTANT R. 2002. Safeguard of a spadefoot toad (*Pelobates fuscus*) population: a French experience. pp. 47-52 in: Ferri V (Ed). *Atti del Terzo Convegno Salvaguardia Anfibi. Progetto ROSPI & Museo cantonale di Storia Naturale di Lugano*. Cogecstre Ediz., Penne.
- FAIRBAIRN DJ. 2007. Introduction: the enigma of sexual size dimorphism. In: Fairbairn DJ, Blanckenhorn WU and Székely T (Eds). *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*, United Kingdom: Oxford University Press, p. 1-12.
- FRECKLETON RP, HARVEY PH & PAGEL M. 2003. Bergmann's rule and body size in mammals. *Am Nat* 161: 821-825.
- GARCÍA-PARÍS M, HERRERO P, MARTÍN C, DORDA J, ESTEBAN M & ARANO B. 1993. Morphological characterization, cytogenetic analysis, and geographical distribution of the Pygmy marbled newt *Triturus marmoratus pygmaeus* (Wolterstorff, 1905) (Caudata: Salamandridae). *Bijdr Dierkd* 63: 31-34.
- GARDNER JL, PETERS A, KEARNEY MR, JOSEPH L & HEINSOHN R. 2011. Declining body size: a third universal response to warming? *Trends Ecol Evol* 26: 285-291.
- GUARINO FM, DE POUS P, CROTTINI A, MEZZASALMA M & ANDREONE F. 2011. Age structure and growth in a population of *Pelobates varaldii* (Anura, Pelobatidae) from northwestern Morocco. *Amphibia-Reptilia* 32: 550-556.
- HALLIDAY TR & TEJEDO M. 1995. Intrasexual selection and alternative mating behaviour. In: Heatwole H and Sullivan BK (Eds). *Amphibian Biology, Social Behaviour*, Chipping Norton: Surrey Beatty, p. 419-468.
- HALLIDAY TR & VERRELL PA. 1986. Sexual selection and body size in amphibians. *Herpetol J* 1: 86-92.
- HALLIDAY TR & VERRELL PA. 1988. Body size and age in amphibians and reptiles. *J Herpetol* 22: 253-265.
- HEMELAAR AMS. 1988. Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes. *J Herpetol* 22: 369-388.
- LECLAIR MH, LECLAIR R & GALLANT J. 2005. Application of skeletochronology to a population of *Pelobates cultripes* (Anura: Pelobatidae) from Portugal. *J Herpetol* 39: 199-207.
- LESKOVAR C, OROMI N, SANUY D & SINSCH U. 2006. Demographic life history traits of reproductive natterjack toads (*Epidalea calamita*) vary between northern and southern latitudes. *Amphibia-Reptilia* 27: 365-375.

- LIAO WB & CHEN W. 2012. Inverse Rensch's rule in a frog with female-biased sexual size dimorphism. *Naturwissenschaften* 99: 427-431.
- LINDSEY CC. 1966. Body sizes of poikilotherm vertebrates at different latitudes. *Evolution* 20: 456-465.
- LOVICH JE & GIBBONS JW. 1992. A review of techniques for quantifying sexual size dimorphism. *Growth Develop Aging* 56: 269-281.
- MARANGONI F. 2006. Variación clinal en el tamaño del cuerpo a escala microgeográfica en dos especies de anuros (*Pelobates cultripes* y *Epidalea calamita*). PhD thesis, University of Seville, Spain.
- MARANGONI F & TEJEDO M. 2008. Variation in body size and metamorphic traits of Iberian spadefoot toads over a short geographic distance. *J Zool* 275: 97-105.
- MARANGONI F, TEJEDO M & GOMEZ-MESTRE I. 2008. Extreme reduction in body size and reproductive output associated with sandy substrates in two anuran species. *Amphibia-Reptilia* 29: 541-553.
- METCALFE NB & MONAHAN P. 2001. Compensation for a bad start: grow now, pay later. *Trends Ecol Evol* 16: 255-260.
- MIAUD C, GUYETANT R & ELMBERG J. 1999. Variations in life-history traits in the common frog *Rana temporaria* (Amphibia: Anura): a literature review and new data from the French Alps. *J Zool* 249: 61-73.
- MICHIMAE H. 2007. Differentiated egg size of the cannibalistic salamander, *Hynobius retardatus*. *J Ethol* 25: 153-158.
- MONNET JM & CHERRY MI. 2002. Sexual size dimorphism in anurans. *P Roy Soc B-Biol Sci* 269: 2301-2307.
- PASCUAL-PONS M, OROMI N, PUJOL-BUXÓ E, FIBLA M, SANUY D & MONTORI A. 2017. Life history traits of a spadefoot toad (*Pelobates cultripes*) population from a semiarid zone in the north east of the Iberian Peninsula. *Herpetol J* 27: 57-61.
- PEABODY CE. 1958. A Kansas drought recorded in growth zones of a bullsnake. *Copeia* 1958: 91-94.
- PLAISTOW SJ, LAPSLEY CT, BECKERMAN AP & BENTON TG. 2004. Age and size at maturity: sex, environmental variability and developmental thresholds. *P Roy Soc B-Biol Sci* 271: 919-924.
- RANTA E, LAURILA A & ELMBERG J. 1994. Reinventing the wheel: analysis of sexual dimorphism in body size. *Oikos* 70: 313-321.
- RAY C. 1960. The application of Bergmann's and Allen's rules to the poikilotherms. *J Morphol* 106: 85-108.
- RICE WR. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223-225.
- ROT-NIKCEVIC I, SIDOROVSKA V, DZUKIC G & KALEZIC ML. 2001. Sexual size dimorphism and life history traits of two European spadefoot toads (*Pelobates fuscus* and *P. syriacus*) in allopatry and sympatry. *Ann Ser Hist Nat* 23: 107-120.
- SAGOR ES, OULLET M, BARTEN E & GREEN DM. 1998. Skeletochronology and geographic variation in age structure in the wood frog, *Rana sylvatica*. *J Herpetol* 34: 469-474.
- SEMLITSCH RD & GIBBONS JW. 1990. Effects of egg size on success of larval salamanders in complex aquatic environments. *Ecology* 71: 1789-1795.
- SHERIDAN JA & BICKFORD D. 2011. Shrinking body size as an ecological response to climate change. *Nat Clim Change* 1: 401-406.
- SHINE R. 1979. Sexual selection and sexual dimorphism in the Amphibia. *Copeia* 1979: 297-306.
- SHINE R. 1992. Relative clutch mass and body shape in lizards and snakes: is reproductive investment constrained or optimized? *Evolution* 46: 828-833.
- SINSCH U, MARANGONI F, OROMI N, LESKOVAR C, SANUY D & TEJEDO M. 2010. Proximate mechanisms determining size variability in natterjack toads. *J Zool* 281: 272-281.
- SINSCH U, OROMI N & SANUY D. 2007. Growth marks in natterjack toad (*Epidalea calamita*) bones: histological correlates of hibernation and aestivation periods. *Herpetol J* 17: 129-137.
- SLATKIN M. 1974. Hedging one's evolutionary bets. *Nature* 250: 704-705.
- STAMPS JA. 1993. Sexual size dimorphism in species with asymptotic growth after maturity. *Biol J Linn Soc* 50: 123-145.
- STĂNESCU F, MARANGONI F, REINKO I & COGĂLNICEANU D. 2016. Life history traits of a Neotropical microhylid (*Dermatonotus muelleri*, Boettger 1885) from the Arid Chaco, Argentina. *Herpetol J* 26: 41-48.
- STATSOFT. 2001. Statistica (data analysis software system), version 6. Tulsa: StatSoft.
- SZÉKELY D, COGĂLNICEANU D, SZÉKELY P, ARMIJOS-OJEDA D, ESPINOSA-MOGROVEJO V & DENOËL M. 2020. How to recover from a bad start: size at metamorphosis affects growth and survival in a tropical amphibian. *BMC Ecology* 20: 24.
- TALAVERA R. 1990. Evolución de Pelobatidos y Peloditidos (Amphibia: Anura): morfología y desarrollo del sistema

esquelético. PhD thesis, Universidad Complutense de Madrid, Madrid, Spain.

VAN KLEUNEN M & FISCHER M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytol* 166: 49-60.

VIGNOLI L, VELLETRANI F, VENDITTI C, LUISELLI L, YADID Y & MACALE D. 2018. An experimental study of the effect of density on the demography and growth of an endangered endemic newt. *Ecological Research* 33: 1039-1048.

VON BERTALANFFY L. 1938. A quantitative theory of organic growth. *Hum Biol* 10: 181-213.

WELLS KD. 2010. *The ecology and behavior of amphibians*. Chicago and London: University of Chicago Press.

WILBUR HM & RUDOLF VHM. 2006. Life-history evolution in uncertain environments: bet-hedging in time. *Am Nat* 68: 398-411.

WOOLBRIGHT LL. 1983. Sexual selection and size dimorphism in anuran amphibians. *Am Nat* 121: 110-119.

**Appendix A. Snout-vent length (SVL, range in mm), age at maturity (AM) and maximum longevity (years), in the four different species of the genus *Pelobates* and *Epidalea calamita* studied by skeletochronology (see Sinsch, 2015 for a complete review of *E. calamita*). ns: not specified. \*: average size. RBD: Reserva Biológica de Doñana.**

Genus / Specie	Locality / elevation (a.s.l.)	Country	Sex	Sample Size	SVL (mm)	AM	Longevity	Source
<b><i>Pelobates</i></b>								
<i>cultripes</i>	Serra da Arriça, 300 m	Portugal	♂	20	43-49	2	8	Leclair et al. (2005)
			♀	40	51-58	2	7	
<i>cultripes</i>	Mas de Melons, 240 m	Spain	♂	27	71.76*	2	5	Pascual-Pons et al. (2017)
			♀	26	76.14*	3	6	
<i>cultripes</i>	Madrid	Spain	♂	32	79.8*	2	12	Talavera (1990)
			♀	32	79.5*	2	12	
<i>cultripes</i>	Doñana, 25.5	Spain	♂	8	55.9*	3	8	Díaz-Paniagua et al. (2005)
			♀	8	60.8*	3	8	
<i>cultripes</i>	Navas, 420 m	Spain	♂	3	75-80	2	4	Marangoni (2006)
			♀	3	85-90	4	6	
<i>cultripes</i>	Pedroso, 395 m	Spain	♂	2	61-88	---	8	Marangoni (2006)
			♀	1	83-86	---	---	
<i>cultripes</i>	Aznalcollar, 130 m	Spain	♂	14	79-99	3	5	This study
			♀	7	86-125	2	6	
<i>cultripes</i>	Abalarío, 63 m	Spain	♂	25	57-82	1	8	This study
			♀	22	61-88	3	7	
<i>cultripes</i>	Bodegonas, 32 m	Spain	♂	27	60-82	2	7	This study
			♀	24	63-97	2	8	
<i>cultripes</i>	RBD, 24.5 m	Spain	♂	21	60-85	2	7	This study
			♀	2	69-75	4	7	
<i>fuscus</i>	Sarre River, 255 m	France	♂	76	Ns	2	7	Eggert & Guyétant (1999)
			♀	25		2	8	
<i>fuscus</i>	Lorraine, NS	France	♂	301	Ns	2	7	Eggert & Guyétant (2002)
			♀	188		2-3	12	
<i>fuscus</i>	Cavolj, 100 m	Serbia	♂	37	41-57	6	16	Rot-Nikcevic et al. (2001)
			♀	22	47-62	3	13	

**Appendix A. Continuation.**

Genus/ Specie	Locality / elevation (a.s.l.)	Country	Sex	Sample Size	SVL (mm)	AM	Longevity	Source
<i>fuscus</i>	Lesino kopovo, 80 m	Serbia	♂	9	36-50	4	14	Rot-Nikcevic et al. (2001)
			♀	11	41-58	4	13	
<i>fuscus</i>	Utrine, 95 m	Serbia	♂	4	41-48	2	6	Rot-Nikcevic et al. (2001)
			♀	15	46-65	4	14	
<i>fuscus</i>	Novara, 150 m	Italy	♂	13	38-47	2	5	Andreone et al. (2004)
			♀	11	46-59	3	5	
<i>fuscus</i>	Constanta, 0.5-1.5 m	Romania	♂	38	37-47	2-5	7	Cogălniceanu et al. (2014)
			♀	34	36-61	2-5	8	
<i>syriacus</i>	Utrine, 95 m	Serbia	♂	14	46-75	3	16	Rot-Nikcevic et al. (2001)
			♀	22	48-80	2	8	
<i>syriacus</i>	FYROM	Macedonia + Bulgaria	♂	16	69-80	6	12	Rot-Nikcevic et al. (2001)
			♀	8	66-79	7	15	
<i>syriacus</i>	Constanta, 0.5-1.5 m	Romania	♂	34	59-93	2-6	10	Cogălniceanu et al. (2014)
			♀	38	55-99	2-4	12	
<i>varaldii</i>	Mamora Forest, 57 m	Morocco	♂	66	44-61	2	7	Guarino et al. (2011)
			♀	20	45-64	2	10	
<b><i>Epidalea</i></b>								
<i>calamita</i>	Navas, 420 m	Spain	♂	26	57-81	1	3	This study; Sinch et al. (2010)
			♀	28	53-85	2	8	
	Pedroso, 395 m	Spain	♂	13	51-88	2	4	This study
			♀	15	54-80	3	6	
	Abalarío, 63 m	Spain	♂	34	52-77	3	7	This study
			♀	24	45-75	2	8	
	Bodegones, 32 m	Spain	♂	23	46-69	2	7	This study
			♀	26	43-61	2	6	
	RBD, 24.5 m	Spain	♂	32	41-72	1	6	This study; Sinch et al. (2010)
			♀	27	39-67	1	4	

**How to cite**

MARANGONI F, TEJEDO M & COGĂLNICEANU D. 2021. Can age and growth patterns explain the geographical variation in the body size of two toad species? *An Acad Bras Cienc* 93: e20190470. DOI 10.1590/0001-3765202120190470.

*Manuscript received on April 22, 2019;  
accepted for publication on August 15, 2019*

**FEDERICO MARANGONI<sup>1,2</sup>**

<https://orcid.org/0000-0001-6242-9222>

**MIGUEL TEJEDO<sup>1</sup>**

<https://orcid.org/0000-0003-4183-184X>

**DAN COGĂLNICEANU<sup>3,4</sup>**

<https://orcid.org/0000-0003-2959-014X>

<sup>1</sup>Department of Evolutionary Ecology, Estación Biológica de Doñana, CSIC, Avda. Américo Vespucio s/n, 41092 Sevilla, Spain

<sup>2</sup>Chelonia Argentina, Virasoro 2804, 3400 Corrientes, Argentina

<sup>3</sup>Chelonia Romania, Pascani 5, sector 6, Bucharest, Romania

<sup>4</sup>Faculty of Natural and Agricultural Sciences, Ovidius University, Constanta, Romania Al. Universității 1, corp B, Constanța 900470, Romania

Correspondence to: **Federico Marangoni**

*E-mail: fedemarangoni@gmail.com*

**Author contributions**

Federico Marangoni conceptualized the study, performed the sampling, laboratory analysis, contributed to data analysis, and wrote the manuscript. Miguel Tejedo acted as a PhD supervisor of Federico Marangoni, acquired the financial resources of this research, designed the study, supervised the project and revised the manuscript. Dan Cogălniceanu contributed to data analysis and manuscript preparation and revision. All authors discussed the results and approved the final version of the manuscript.

