

## Age and Body Size in Populations of Two Syntopic Spadefoot Toads (Genus *Pelobates*) at the Limit of Their Ranges

DAN COGĂLNICEANU,<sup>1</sup> DANIELA ROȘIORU,<sup>1,3</sup> PAUL SZÉKELY,<sup>1,5</sup> DIANA SZÉKELY,<sup>1</sup> ELENA BUHACIUC,<sup>1</sup> FLORINA STĂNESCU,<sup>1</sup> AND CLAUDE MIAUD<sup>4,5</sup>

<sup>1</sup>University Ovidius Constanța, Faculty of Natural Sciences, Al. Universității 1, corp B, Constanța, Romania

<sup>2</sup>National Institute for Marine Research and Development “Grigore Antipa”, Blvd. Mamaia nr. 300, Constanța, Romania

<sup>3</sup>UMR 5553 LECA, Université de Savoie, Laboratoire d'Ecologie Alpine, Le Bourget du Lac, France

<sup>5</sup>UMR 5175 CEFE, Ecole Pratique des Hautes Etudes, Laboratoire Biogéographie et Ecologie des Vertébrés, 1919 route de Mende, Montpellier, France

**ABSTRACT.**—Age and size at sexual maturity are major life-history traits that affect growth rate and reproductive output. The differences in these life-history traits can be better assessed in syntopic populations of related species, where environmental variability in time and space is removed. The Spadefoot Toads (*Pelobates* spp.) are highly specialized burrowing and nocturnal species with a narrow ecological niche. We tested if age-related parameters are responsible for sexual size dimorphism and size differences in two syntopic populations from the Danube Delta Biosphere Reserve (Romania). The two populations differed significantly in size and body mass, and showed distinct sexual dimorphism, with females significantly larger in *P. fuscus*, and males slightly larger in *P. syriacus*. We also found differences in age structure: *P. fuscus* was on average 5.0 yr old, whereas *P. syriacus* was on average 7.4 yr old. Both species reached sexual maturity at a similar age. 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 between these syntopic populations. Our results suggest that growth allocation between species and sexes cannot be explained solely on the basis of age-related parameters.

A central assumption of life-history theory is that the evolution of component traits is determined in part by trade-offs between these traits (Roff et al., 2006). Age and size at sexual maturity are key life-history traits that affect growth rate, fecundity, and survival (Roff, 1992). Animals with indeterminate growth (e.g., ectotherms, in which growth continues past maturation) experience a trade-off in resource allocation between reproduction and growth during their entire life (Heino and Kaitala, 1999).

Age and size at sexual maturity determine both how fast individuals within a population can start reproducing but also how large their reproductive output will be, since fecundity is often closely associated with body size (Plastow et al., 2004). Although increased body size is usually accompanied by increased fecundity in females, this relation can be weakened by the existence of trade-offs between current and future reproduction events (Heino and Kaitala, 1999).

Syntopic populations of related species are good study systems since they are subject to similar environmental conditions, removing differences such as food availability and temperature. For example, in ectotherms, maturation occurs later and at smaller sizes when growth rates are lowered by reduction in food availability, but they mature later and at larger sizes when growth rate is lowered by decreased temperature (Berrigan and Charnov, 1994).

Sexual dimorphism describes intraspecific differences in size, shape, or traits between males and females (Fairbairn, 2007). Sexual size dimorphism (SSD) evolves as the indirect consequence of the different reproductive roles of males and females (Andersson, 1994) and is a population property emerging from sexual differences in life history (Halliday and Verrell, 1988; Monnet and Cherry, 2002). SSD is shaped by fecundity selection in female-biased SSD (Roff, 1992) and sexual selection, i.e., male–male competition or female choice in male-biased SSD (Andersson, 1994). In ectotherms such as anurans, there were several attempts to explain variations in SSD: male–male

interactions (Shine, 1979), female choice for larger males (Halliday and Verrell, 1988), or differences in age structure between sexes that lead to differences in body size (Miaud et al., 1999; Monnet and Cherry, 2002; Liao and Chen, 2012).

The long-term coexistence of related species within overlapping ranges is unlikely in the absence of ecological differentiation (Weissing et al., 2011), but sexual selection can provide ways to reduce niche overlap between species, allowing long-term coexistence (M'Gonigle et al., 2012). The aim of this work is to study the relationship between coexistence and SSD in two related amphibian species.

Two related Spadefoot Toad species (*Pelobates* spp.) occur in southeastern Europe (*Pelobates syriacus* and *Pelobates fuscus*), but their ranges overlap only in the Balkan Peninsula (Džukić et al., 2008). Adult *P. syriacus* are larger than *P. fuscus* and the SSD is female-biased in *P. fuscus*, whereas males are of similar size or larger than females in *P. syriacus* (Rot-Nikčević et al., 2001; Ugurtaş et al., 2002), a situation reported in only 10% of anuran species (Shine, 1979). We report life-history traits (age, size, and growth) to test whether age-related parameters can explain body size and SSD differences in the two species living in two syntopic populations. At a finer scale we focused on how these traits change when in syntopy, and tested if they differ in these populations from allopatric populations in the rest of the species range.

### MATERIALS AND METHODS

**Study Species.**—The Spadefoot Toads (*Pelobates* spp.) are highly specialized burrowing and nocturnal species with a narrow ecological niche (Nöllert, 1990). Because of their secretive behavior (nocturnal activity, weak underwater breeding call, cryptic coloration), Spadefoot Toads' life histories are not well documented and overall there is a scarcity of information about the genus. Two related species occur in southeastern Europe: *P. syriacus* and *P. fuscus*. Although their ranges are mostly disjoint, they overlap in the Balkan Peninsula, along the lower course of the Danube River and the western coast of the Black Sea. Both species reach the limits of their ranges here: southern limit for *P.*

<sup>3</sup>Corresponding Author. E-mail: szpaul@gmail.com  
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TABLE 1. SVL, body mass, age, and age at sexual maturity in two syntopic populations of Spadefoot Toads (genus *Pelobates*) from Grindul Lupilor (Danube Delta Biosphere Reserve). The data presented are average values with standard deviation; in brackets the minimum and maximal values. Sample size (N) is indicated below each parameter.

Species	Sex	SVL (mm)	Body mass (g)	Age (yr)	Age at sexual maturity (yr)
<i>Pelobates fuscus</i> n = 77	M	41.7 ± 2.4 (37.6–47.3) n = 43	7.8 ± 1.4 (5.5–11.2) n = 43	5.1 ± 1.2 (2–7) n = 38	3.5 ± 0.7 (2–5) n = 25
	F	47.7 ± 6.2 (36.9–61.5) n = 34	13.0 ± 7.2 (5.2–33.5) n = 34	5.0 ± 1.2 (3–8) n = 34	3.1 ± 0.7 (2–5) n = 25
<i>Pelobates syriacus</i> n = 76	M	76.5 ± 10.8 (59.3–93.7) n = 37	51.5 ± 20.9 (21.2–91.9) n = 37	7.5 ± 1.5 (4–10) n = 34	3.5 ± 0.9 (2–6) n = 24
	F	74.4 ± 12.0 (55.4–99.5) n = 39	56.8 ± 25.0 (19.2–118.1) n = 39	7.3 ± 1.8 (3–12) n = 38	3.2 ± 0.7 (2–4) n = 23

*fuscus* and northern limit for *P. syriacus* (Džukić et al., 2008). It was suggested that the limits of their ranges might be restricted by competition between the related species (Tarkhnishvili et al., 2009), but this hypothesis was not tested. The ranges of the other two species of *Pelobates* do not overlap: *P. varaldii* occurs in a restricted area in North Africa (de Pous et al., 2012), whereas *P. cultripes* occurs on the Iberian Peninsula and in southern France (Garcia-Paris et al., 2004).

**Site Location.**—The study area is located in a lagoon system within the Danube Delta Biosphere Reserve (Romania), between Grindul Lupilor, a natural sand levee (44°37'N, 28°48'E) and the Village of Vadu, Constanta County (44°27'N, 28°45'E). The terrestrial habitats are partly salinized sandy soils, covered with steppe vegetation, located within an altitudinal range of 0.5–1.5 m above sea level (a.s.l.). The water level within this large wetland is highly variable as it depends on Danube water flow, which ranges between 1,500 and 19,000 m<sup>3</sup>/s (Cogălniceanu, 2012). Seven other amphibian species are present in the study area: *Lissotriton vulgaris*, *Triturus dobrogicus*, *Pelophylax* kl. *esculentus*, *P. ridibundus*, *Bombina bombina*, *Bufo viridis*, and *Hyla arborea*.

**Sampling and Analysis.**—Spadefoot Toads (153 adults: 77 *P. fuscus* and 76 *P. syriacus*, and 18 juveniles) were collected in spring in 2010 and 2011. Since these toads are strictly nocturnal, we carried out all field activities at night. The animals were collected and stored in plastic buckets, briefly blotted on filter paper, mass determined with a precision of 0.01 g on a portable electronic balance (Kern PCB), and their snout–vent length (SVL) measured with digital calipers with a 0.1-mm precision. The second phalange of the left forearm was removed and stored in 70% alcohol for an assessment of age through skeletochronology (sensu Castanet and Smirina [1990]; modified following Rozenblut and Ogielska [2005]). We decalcified the second phalange for 3 h with 5% HNO<sub>3</sub>, followed by inclusion in TissueTek, freezing, and sectioning with a Tehsys CR 3000 cryotome at 16 μm. The cross-sections were stained with Ehrlich's hematoxylin for 3 h. Cross-sections were photographed using an Olympus CX 31 microscope and Quick Photo Micro 2.3 software. We estimated age from the lines of arrested growth (LAGs), after correction for resorption using cross-sections from juveniles. The distance between two LAGs is an indicator of individual growth in a given age (Kleinenberg and Smirina, 1969), and a pattern of decreasing intervals between LAGs after a few years is thought to indicate the onset of sexual maturity, with resources being reallocated from growth to reproduction (Smirina, 1994).

**Data Analysis.**—Normality of the data was tested with Shapiro–Wilk's test. Normally distributed data were compared using analysis of variance (ANOVA). Where the normal distribution assumption was violated, we tested for differences

using Kruskal–Wallis one-way ANOVA on ranks. We did a multiple linear regression with either SVL or body mass as dependent variables and age, age at sexual maturity, and year as independent variables for both species and sexes. Data were analyzed using SigmaPlot version 12.0 (Systat Software Inc.).

To test if the pattern observed in our studied populations is valid outside the area of range overlap we compared the SVL of males with the SVL of females on the basis of published data (48 populations) and our own data (9 populations) (see Appendix 1).

We considered growth to be an incremental change in size (Busacker et al., 1990) and the average body growth of toads can

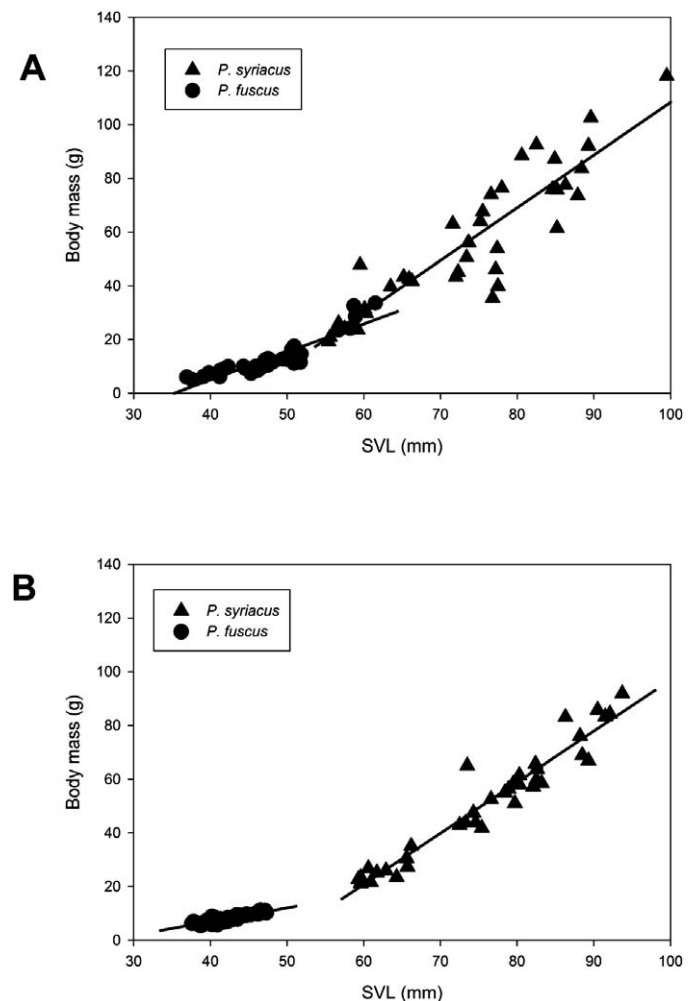


FIG. 1. SVL and body mass relationships in the two species of Spadefoot Toads (genus *Pelobates*) in females (A) and males (B).

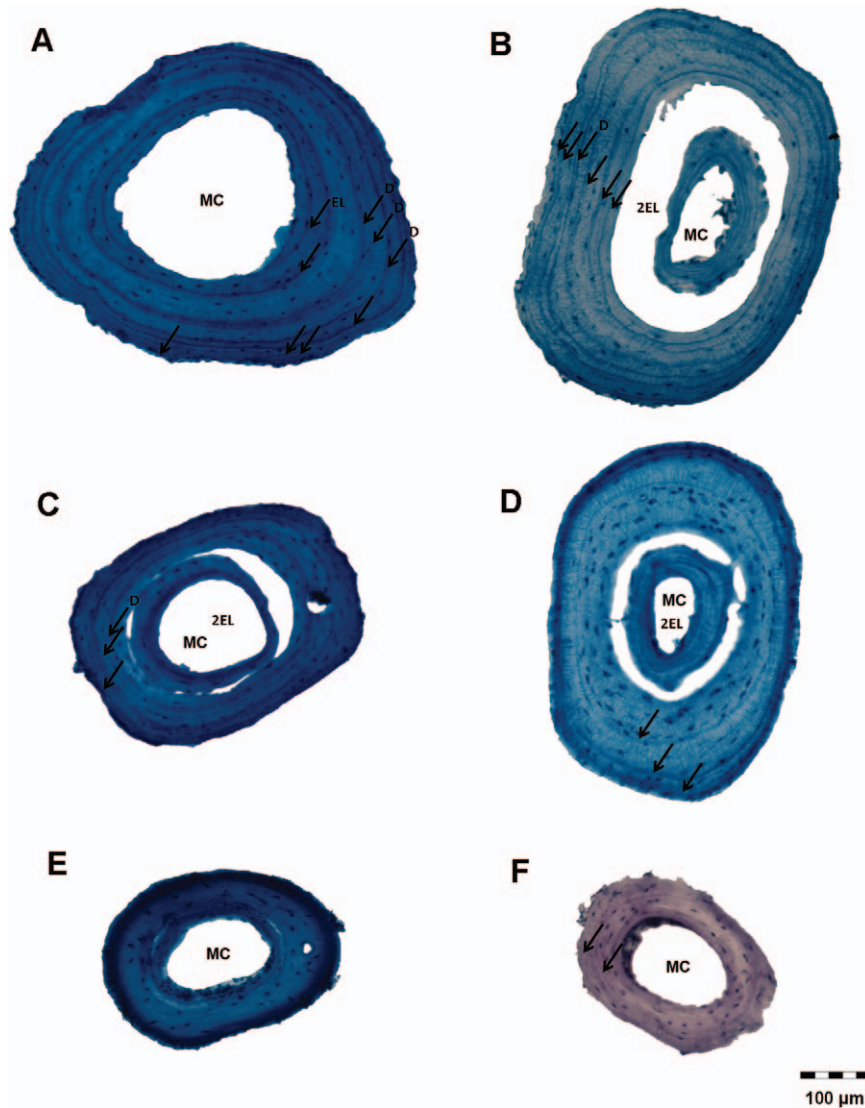


FIG. 2. Cross-sections through the diaphysis of the phalange of *Pelobates syriacus* and *P. fuscus*. The black arrows mark the number of LAGs; EL, eroded LAG; D, double LAG; MC, marrow cavity. The juveniles were used to mark endosteal erosion and the missing LAG (EL), necessary for the correction of the age estimation. (A) *P. syriacus* female at 9 yr old and 4 yr sexual maturity, with one partly EL and three D. (B) *P. syriacus* male at 8 yr old and 4 yr sexual maturity, with two EL and one D. (C) *P. fuscus* female at 5 yr old and 4 yr sexual maturity, with two EL and two D. (D) *P. fuscus* male at 5 yr old and 4 yr sexual maturity, with two EL. (E) *P. syriacus* juvenile at 0.5 yr old. (F) *P. fuscus* juvenile at 2 yr old.

be described by the von Bertalanffy (von Bertalanffy, 1957) growth equation:

$$S_t = S_m - (S_m - S_0) \times \exp(-K(t - t_0))$$

where  $t$  = number of growing seasons experienced (age);  $t_0$  = age at metamorphosis (proportion of the growing season already elapsed at metamorphosis, e.g., 0.25 = 3 mo);  $S_t$  = average body size after having experienced  $t$  growing seasons;  $S_m$  = average maximal body size;  $S_0$  = average body size at metamorphosis. We used the SVL at metamorphosis from (Székely, 2010): *P. fuscus* 28.2 mm and *P. syriacus* 28.8 mm.  $K$  = growth coefficient or rate at which  $S_t$  is being reached, defines the shape of the curve.  $S_t$  is the estimated average of the attainable maximum body length.

The starting point of the growth curve was at metamorphosis, i.e., growth during the aquatic larval stage was not considered. SVL<sub>max</sub> and  $K$  and their confidence intervals were estimated by nonlinear least-squares regression. Two estimated SVL<sub>max</sub> and  $K$  values were considered to be significantly different at the 0.95

level when their confidence intervals did not overlap. All tests were performed with the Statistica 6.0/W software package (Statsoft Inc.).

SSD was calculated on adult body length by using the sexual dimorphism index (SDI, Lovich and Gibbons, 1992). SDI = ratio of mean SVL of the larger sex divided by mean SVL of the smaller sex, with the result defined arbitrarily as positive when females are larger than males.

## RESULTS

*Body Size, Mass, and SSD.*—*P. fuscus* females were larger than males in both SVL and body mass (Kruskal-Wallis,  $P < 0.001$ ). The SSD, calculated with the SDI, was 1.14 for the body size and 1.67 for the mass. In *P. syriacus*, SVL and body mass did not differ between sexes (Kruskal-Wallis,  $P = 0.27$  and  $P = 0.32$  respectively). The SDI was  $-1.03$  and  $-1.10$  for the body size and the mass respectively (Table 1).

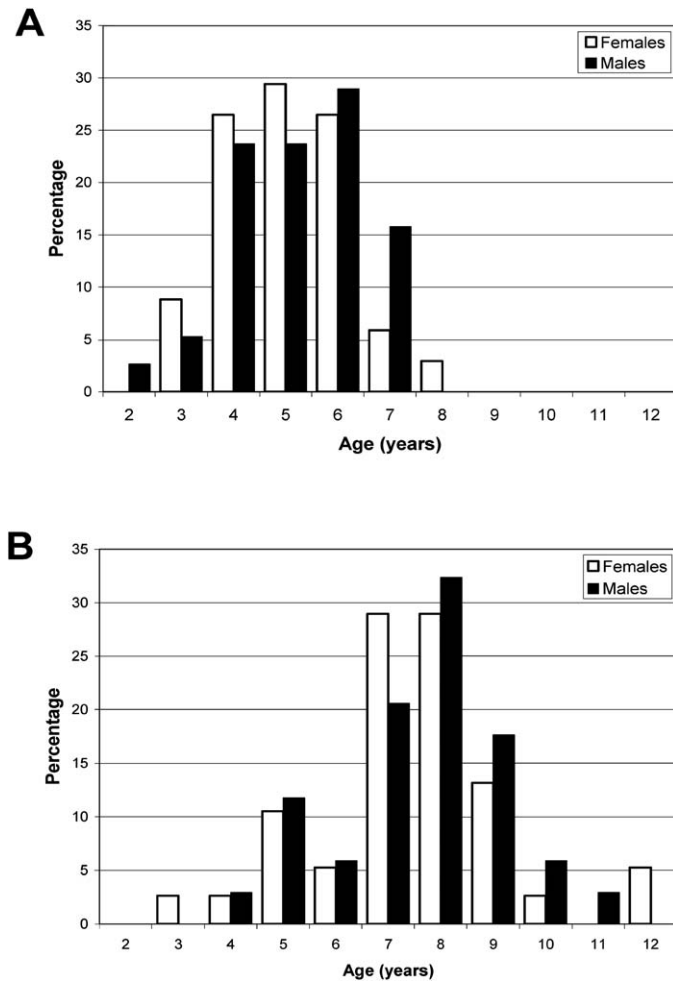


FIG. 3. Population age structure in males and females of the two studied species. Males are represented by solid bars and females are represented by open bars. *Pelobates fuscus* and *P. syriacus* are represented by (A) respectively (B).

The two Spadefoot Toad species differed in size (*t*-test on log-transformed data: females  $t = 12.53$ ,  $df = 71$ ,  $P < 0.001$ ; males  $t = 24.93$ ,  $df = 78$ ,  $P < 0.001$ ) and body mass (*t*-test on log-transformed data: females  $t = 13.53$ ,  $df = 71$ ,  $P < 0.001$ ; males  $t = 24.02$ ,  $df = 78$ ,  $P < 0.001$ ) (Table 1). The ratio *fuscus:syriacus* differed between size and mass: SVL females 0.7, males 0.63; body mass females 0.26, males 0.21 (Table 1). The correlation between SVL and body mass showed differences in slope between the two species, with body mass increasing faster with SVL in *P. syriacus* as compared with *P. fuscus* (Fig. 1).

**Age-Related Parameters.**—LAGs were observed in the cross-sections of the periostic bone of phalanges (Fig. 2). Endosteal resorption was observed in 71 and 53 of the cross-sections in *P. fuscus* and *P. syriacus* respectively. This resorption reached the

first (innermost) LAG in 12 (15.6%) and 41 individuals (53.9%) in *P. fuscus* and *P. syriacus* respectively, and the second LAG in 59 (76.6%) and 12 individuals (15.8%) in *P. fuscus* and *P. syriacus* respectively. Age of adults could be assessed in 72 *P. fuscus* (93.5%) and 72 *P. syriacus* (94.7%) (Fig. 2). The average age differed significantly between the two species (Kruskal–Wallis,  $P < 0.001$ ), *P. syriacus* was older on average and had greater longevity (Table 1, Fig. 3). Age did not differ between sexes in either species (*P. fuscus* ANOVA,  $F_{1,70} = 0.107$ ,  $P = 0.74$ ; *P. syriacus* Kruskal–Wallis,  $P = 0.64$ ).

Age at maturity can be defined as the youngest adult age observed, e.g., 2 and 3 yr old in male and female *P. fuscus* respectively, and 4 and 3 yr old in male and female *P. syriacus* respectively (Table 1). A pattern of decreasing intervals between LAGs indicating the onset of sexual maturity was observed clearly in 50 *P. fuscus* (66%) and 47 *P. syriacus* (62%). On the basis of this pattern of LAGs we estimated that the two species reached sexual maturity at a similar age of 3 yr (Kruskal–Wallis,  $P = 0.13$ , Table 1). Within species, *P. fuscus* males matured later than females (Kruskal–Wallis,  $P < 0.05$ ), whereas no significant differences were observed in *P. syriacus*.

**Growth.**—The von Bertalanffy's growth model fitted correctly the relation between age and SVL in both species (Table 2; coefficients of correlation from 0.52 to 0.86). In *P. fuscus* the growth coefficient ( $K$ ) was higher in males than in females, whereas the maximum asymptotic size (SVL<sub>max</sub>) was higher in females than in males. The two growth curves differed before minimum age at sexual maturity (i.e., 2 and 3 yr in males and females respectively). The flattened growth curve in males differed strongly from the shape of the growth curve for females (Fig. 4), supporting the observed SSD (SDI = 1.14) in *P. fuscus*. On the other hand, no difference was observed in growth parameters between male and female *P. syriacus* (Table 2 and Fig. 4), supporting the observed absence of SSD (SDI = -1.03) in this species.

**Relationships among Life-History Traits.**—The multiple linear regression showed that body size (i.e., SVL and body mass) in *P. fuscus* females were predicted significantly by age at sexual maturity (SVL:  $n = 26$ ,  $R^2 = 0.47$ ,  $P = 0.27$ ; body mass:  $n = 26$ ,  $R^2 = 0.47$ ,  $P = 0.01$ ). On the other hand, age at sexual maturity had no predictive value for SVL or body mass in *P. fuscus* males ( $P > 0.05$ ). A similar analysis in *P. syriacus* identified year of capture (i.e., 2010 or 2011) as a good predictor of SVL and body mass ( $P < 0.05$ ). We then compared size and age of animals on the basis of the year of capture. There was a difference in SVL between years in females (ANOVA,  $F_{1,37} = 3.34$ ,  $P = 0.004$ ), but not in body mass (ANOVA,  $F_{1,37} = 9.53$ ,  $P = 0.076$ ). Similarly, males differed between years in both SVL (ANOVA,  $F_{1,34} = 11.78$ ,  $P = 0.002$ ) and body mass (ANOVA,  $F_{1,34} = 9.82$ ,  $P = 0.004$ ). Both sexes had higher SVL and body mass in 2011 compared with 2010. Age structure did not differ between 2010 and 2011 in either males or females (Kruskal–Wallis: males  $P = 0.14$ , females  $P = 0.46$ ).

TABLE 2. Growth (estimated from SVL and body mass) in two syntopic populations of Spadefoot Toads (genus *Pelobates*) from Grindul Lupilor (Danube Delta Biosphere Reserve). CI = confidence interval,  $K$  = growth coefficient, SVL<sub>max</sub> = asymptotic body size,  $W_{max}$  = asymptotic body mass,  $R$  = correlation coefficient, Var = percentage of variance explained by the von Bertalanffy's model.

Species	Sex	$K \pm CI$	SVL <sub>max</sub> $\pm CI$	$R$	Var (%)	$K \pm CI$	$W_{max} \pm CI$	$R$	Var (%)
<i>P. fuscus</i>	M	0.39 $\pm$ 0.04	44.0 $\pm$ 0.45	0.52	27.5	0.32 $\pm$ 0.04	9.0 $\pm$ 0.28	0.36	15.39
	F	0.12 $\pm$ 0.01	71.1 $\pm$ 2.05	0.61	37.4	0.10 $\pm$ 0.01	30.2 $\pm$ 2.93	0.47	22.61
<i>P. syriacus</i>	M	0.30 $\pm$ 0.03	83.6 $\pm$ 1.82	0.86	75.6	0.16 $\pm$ 0.02	74.7 $\pm$ 4.4	0.74	54.60
	F	0.26 $\pm$ 0.03	83.02 $\pm$ 1.86	0.84	70.2	0.10 $\pm$ 0.01	110.1 $\pm$ 5.9	0.77	59.82

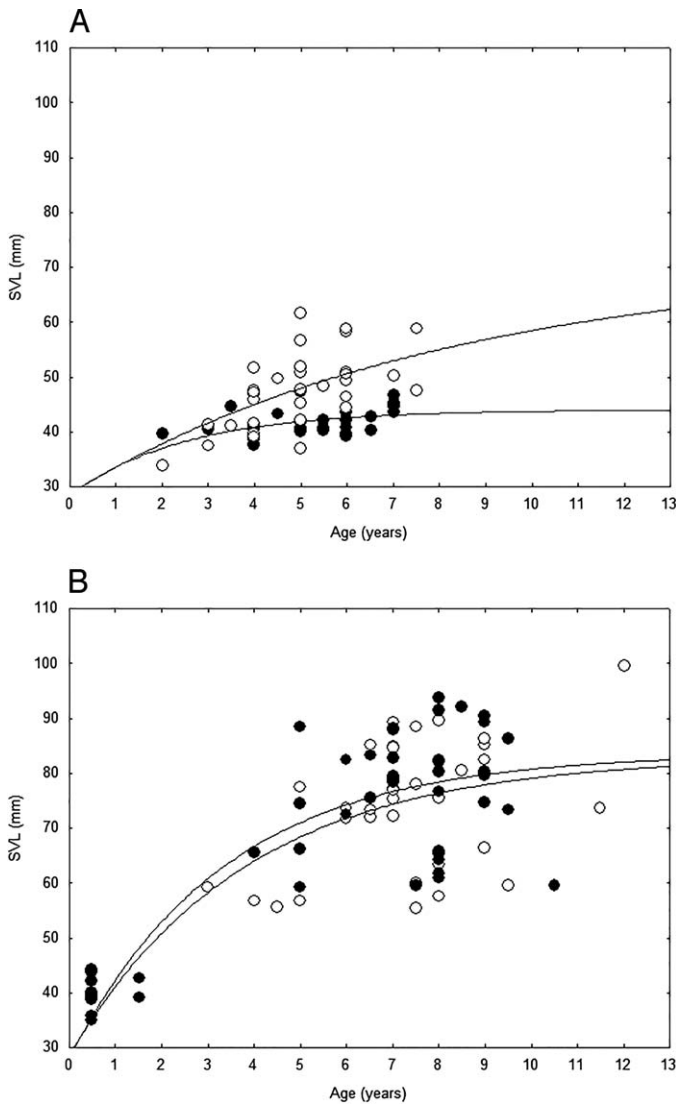


FIG. 4. Growth curves in *Pelobates fuscus* (A) and *P. syriacus* (B). We used individuals of the two studied species from syntopic populations in southeastern Europe.  $x$ -axis = age in years assessed by skeletochronology;  $y$ -axis = SVL in millimeters. Growth curves were fitted to von Bertalanffy's growth equation. Parameters are available in Table 2. Open dots, females; black dots, males.

#### DISCUSSION

*Life-History Traits of Two Species at the Range Limit.*—Both Spadefoot Toad species differ in their growth rate before and after reaching sexual maturity. In *P. syriacus* and in *P. fuscus* males, growth rate was higher before reaching sexual maturity, whereas in *P. fuscus* females' growth rate continued at a similar rate, thus explaining the SSD in this species. At metamorphosis the juveniles of the two species did not differ in SVL (mean  $\pm$  SD: *P. fuscus* 28.2  $\pm$  2.2 mm, *P. syriacus* 28.8  $\pm$  2.1 mm; ANOVA  $F_{1,138} = 3.13$ ,  $P = 0.079$ ) (Székely, 2010). As adults the differences in size are significant and the SVL ratio of *P. fuscus* compared with *P. syriacus* changes from 98% in metamorphs to 65% in adults. The percentage of larval growth of the total growth is 64% in *P. fuscus* males, 39% in *P. fuscus* females, and 34.7% in *P. syriacus*. The shifts in body mass are even more impressive: metamorphs differ significantly between species in body mass (*P. fuscus* 3.3  $\pm$  0.6 g, *P. syriacus* 3.7  $\pm$  0.8 mm; ANOVA  $F_{1,138} = 9.77$ ,  $P = 0.002$ )

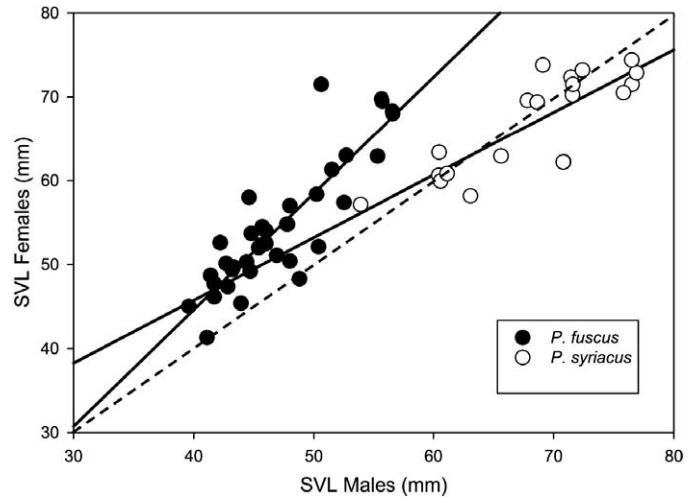


FIG. 5. Sexual size dimorphism of SVL for the two studied species. We assessed the sexual size dimorphism of SVL in 37 populations of *Pelobates fuscus* and 20 populations of *P. syriacus* on the basis of published and our own data (see Appendix 1).

(Székely, 2010), and the body mass ratio of *P. fuscus* compared with *P. syriacus* changes from 89% as metamorphs to 24% as adults. These results indicate clearly that *P. syriacus* has a higher growth rate and achieves larger body size than *P. fuscus*, despite reaching sexual maturity at a similar age. This is consistent with previous studies in the Smooth Newt (*L. vulgaris*) (Halliday and Verrell, 1988), the Common Frog (*Rana temporaria*) (Miaud et al., 1999), and the Natterjack Toad (*Bufo [Epidalea] calamita*) (Sinsch et al., 2010), where growth rate before reproduction is a more significant source of variance in body size than is age.

The age parameters of all four species of the genus *Pelobates* have been studied previously in several populations (Table 3). Several patterns can be inferred from the available data; for example, age at sexual maturity was about 2–3 yr, independent of size. Apart from the study of Rot-Nikčević et al. (2001), other studies reported a mean age of 5 yr or less, and a low longevity, usually below 10 yr. Also, apart from *P. syriacus*, in the other three *Pelobates* species females are larger than males. A variety of studies has shown that age and size at sexual maturity can change plastically in response to environmental conditions (Day and Rowe, 2002), and this could explain the variability in a population. For example, both size and temperature affect metabolic rate of ectotherms through different mechanisms, and smaller body sizes may compensate for lower metabolic rates caused by lower ambient temperatures (Makarieva et al., 2005).

The study site is often subjected to rapid changes in water level and prolonged droughts, making the environment unpredictable for amphibians and affecting reproductive success. Both theoretical and empirical studies have demonstrated that environmental uncertainty can have a strong influence on the evolution of life histories (e.g., Lytle and Poff, 2004; Wilbur and Rudolf, 2006). Such an unpredictable environment diminishes the returns from reproductive investment and promotes indeterminate growth (Heino and Kaitala, 1999). A generally accepted assumption is that an increase in one life-history trait that by itself increases fitness is countered by a change in another trait that decreases fitness (e.g., survival decreases with an increase in fecundity) (Roff, 1992).

TABLE 3. Age structure and SVL in the four species of Spadefoot Toads (genus *Pelobates*).

Species	Location	Sample size		Average SVL (mm)		Age at sexual maturity (yr)		Mean age (yr)		Longevity (yr)		Source
		M	F	M	F	M	F	M	F	M	F	
<i>P. cultripes</i>	Portugal, Serra da Arrica, 300 m a.s.l.	20	40	52.8	54.4	2–3	2–3	4.5	5	8	7	(Leclair et al., 2005)
<i>P. varaldii</i>	Morocco, Mamora Forest, 57 m a.s.l.	66	20	51.7	52.8	2	2	4.5	4.7	7	10	(Guarino et al., 2011)
<i>P. syriacus</i>	Serbia, Utrine, 95 m a.s.l.	14	22	61.1	60.8	2–3	2–3	6	4.6	16	6	(Rot-Nikčević et al., 2001)
<i>P. syriacus</i>	Macedonia and Bulgaria	16	8	75.8	70.5	-	-	9.4	9.6	12	15	(Rot-Nikčević et al., 2001)
<i>P. fuscus</i>	Serbia, Utrine, 95 m a.s.l.	4	15	44.8	53.7	2	2–4	5	7.9	6	14	(Rot-Nikčević et al., 2001)
<i>P. fuscus</i>	Serbia, Cavolj, 100 m a.s.l.	37	22	46	54	2–4	2–4	10.2	8.3	16	13	(Rot-Nikčević et al., 2001)
<i>P. fuscus</i>	Serbia, Lesino Kopovo, 80 m a.s.l.	9	11	43.9	45.4	3–5	2–5	7.9	8	14	13	(Rot-Nikčević et al., 2001)
<i>P. fuscus</i>	Italy, Novara, 150 m a.s.l.	13	11	45.7	54.5	2	3	3.4	4	5	5	(Andreone et al., 2004)
<i>P. fuscus</i>	France, Sarre River, 255 m a.s.l.	87	29	-	-	2	2	2.8	5	7	8	(Eggert and Guyétant, 1999)
<i>P. fuscus</i>	France, Lorraine	242	192	-	-	2	2–3	4.4	5	7	12	(Eggert and Guyétant, 2002)

*Species in Syntopy.*—The plot of male vs. female average SVL from 57 populations of both Spadefoot Toad species (Appendix 1) indicated a significant relationship between average male and female size across populations (*P. fuscus*:  $n = 37$ ,  $R^2 = 0.75$ ,  $P < 0.001$ ; *P. syriacus*:  $n = 20$ ,  $R^2 = 0.68$ ,  $P < 0.001$ ). The regression lines differ in slope, indicating that small increments in average male size correspond to higher increments in size in average size of *P. fuscus* females. Populations positioned above the line indicate populations where males are smaller than females, those situated below the line indicate populations where males are larger than females, and those situated along or close to the dotted line indicate populations without SSD (Fig. 5). Fecundity selection in females and sexual selection in males are considered the major evolutionary forces that select for larger body size in most organisms (Blanckenhorn, 2000). Halliday and Verrell (1988) suggested that female choice of larger males favors males that have shown rapid juvenile growth, and not just older age. This might explain the rapid growth pattern of *P. syriacus* males, which might be advantageous for reproduction. 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 and Sibly, 2006). Analyzing a data set from 33 urodele species, Zhang and Lu (2013) concluded that higher longevities in one sex are mediated by prolonged maturation that allows that sex to grow longer and get larger, with a weak contribution of growth rate to body size. The significant differences in size and growth rates between the two Spadefoot

Toad species are an indicator of ecological differentiation that reduces interspecific competition.

Our study shows that differences in size between species and sexes cannot be explained solely on the basis of age-related parameters. Different growth rates before sexual maturity and differences in energy allocation between growth and reproduction after sexual maturity are in part responsible for the differences in size between these two syntopic populations. These facilitate an ecological differentiation that might explain to a certain extent the coexistence of the two species.

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APPENDIX 1. Average SVL of males and females from 57 populations of Spadefoot Toads and the SDI computed for each population.

No.	Species	Location	Country	Male sample size	SVL Males	Female sample size	SVL Females	SDI	Source
1	<i>Pelobates fuscus</i>	Utrine	Serbia	5	44.79	26	53.71	1.20	(Rot-Nikčević et al., 2001)
2	<i>P. fuscus</i>	Cavolj	Serbia	39	46	28	54.02	1.17	(Rot-Nikčević et al., 2001)
3	<i>P. fuscus</i>	Lesino Kopovo	Serbia	16	43.93	27	45.38	1.03	(Rot-Nikčević et al., 2001)
4	<i>P. fuscus</i>	Vienna	Austria		42.69		50.12	1.17	(Schmidt et al., 2012)
5	<i>P. fuscus</i>	Calafat	Romania	13	44.7	7	49.2	1.10	Own unpublished data
6	<i>P. fuscus</i>	Vadu	Romania	31	41.7	15	46.14	1.11	Own unpublished data
7	<i>P. fuscus</i>	Grindul Lupilor	Romania	43	41.7	34	47.7	1.14	Present study
8	<i>P. fuscus</i>	Viile and Băciu Lake	Romania	3	51.5	9	61.3	1.19	Own unpublished data
9	<i>P. fuscus</i>	Sălicea	Romania	233	55.3	143	62.9	1.14	Own unpublished data
10	<i>P. fuscus</i>	Insula Mică a Brăilei	Romania	8	41.4	2	48.7	1.18	Own unpublished data
11	<i>P. fuscus</i>	Străuleți	Romania	10	50.6	1	71.5	1.41	Own unpublished data
12	<i>P. fuscus</i>		Netherlands	2	48	10	50.4	1.05	(Oomen, 1966)
13	<i>P. fuscus</i>		Germany	7	55.65	2	69.75	1.25	(Hildenhagen et al., 1981)
14	<i>P. fuscus</i>		Germany	5	56.56	2	68.3	1.21	(Nöllert, 1990)
15	<i>P. fuscus</i>		Germany	14	48	8	57	1.19	(Döhring, 1936)
16	<i>P. fuscus</i>	Brandenburg	Germany	100	42.2	25	52.6	1.25	(Nöllert, 1990)
17	<i>P. fuscus</i>		Germany	10	42.83	8	47.37	1.11	(Nöllert, 1990)
18	<i>P. fuscus</i>		Germany	29	47.75	21	54.8	1.15	(Nöllert, 1990)
19	<i>P. fuscus</i>		Poland	161	44.4	157	50.3	1.13	(Andrzejewski et al., 1977)
20	<i>P. fuscus</i>		Ukraine	20	52.5	18	57.39	1.09	(Sčerbanj, 1980)
21	<i>P. fuscus</i>	Mecklenburg-Vorpommern	Germany	61	46	37	52.5	1.14	(Nöllert and Günther, 1996)
22	<i>P. fuscus</i>	Niedersachsen	Germany	545	52.7	223	63	1.20	(Nöllert and Günther, 1996)
23	<i>P. fuscus</i>	Nordrhein-Westfalen	Germany	7	55.7	2	69.5	1.25	(Nöllert and Günther, 1996)
24	<i>P. fuscus</i>	Klewen	Germany	5	56.6	2	68	1.20	(Nöllert and Günther, 1996)
25	<i>P. fuscus</i>	Sachsen-Anhalt	Germany	29	47.8	21	54.8	1.15	(Nöllert and Günther, 1996)
26	<i>P. fuscus</i>	Niedersachsen (Braunschweig)	Germany	866	43.3	289	49.7	1.15	(Tobias, 1997)
27	<i>P. fuscus</i>	Vienna	Austria	427	39.6	199	45	1.14	(Wiener, 1997)
28	<i>P. fuscus</i>		Holland	7	45.4	21	52	1.15	(Ottburg et al., 2005)
29	<i>P. fuscus</i>		Holland	7	44.6	3	58	1.30	(Ottburg et al., 2005)
30	<i>P. fuscus</i>		Holland	14	48.8	14	48.3	-1.01	(Ottburg et al., 2005)
31	<i>P. fuscus</i>		Holland	28	46.9	38	51.1	1.09	(Ottburg et al., 2005)
32	<i>P. fuscus</i>		Holland	16	43.2	7	49.4	1.14	(Ottburg et al., 2005)
33	<i>P. fuscus</i>		Holland	23	41.1	9	41.3	1.00	(Ottburg et al., 2005)
34	<i>P. fuscus insubricus</i>	Ivrea, Turin	Italy	70	45.7	73	54.5	1.19	(Andreone et al., 2004)
35	<i>P. fuscus insubricus</i>	Ivrea, Turin	Italy	22	50.22	11	58.36	1.16	(Andreone and Pavignano, 1988)
36	<i>P. fuscus insubricus</i>	Varese	Italy		50.38		52.12	1.03	(Gentili and Scali, 2001)
37	<i>P. fuscus insubricus</i>	Asti Province	Italy	12	45.6	2	52.3	1.15	(Mercurio and Vigni, 2007)
38	<i>P. syriacus</i>	Deliblato	Serbia	29	60.44	32	60.62	1.00	(Ugurtaş et al., 2002)
39	<i>P. syriacus</i>	Ivanovo	Serbia	4	53.89	9	57.13	1.06	(Ugurtaş et al., 2002)
40	<i>P. syriacus</i>	Ovce Pole	Macedonia	4	71.45	3	72.34	1.01	(Ugurtaş et al., 2002)
41	<i>P. syriacus</i>	Monospitovo	Macedonia	15	60.47	24	63.37	1.05	(Ugurtaş et al., 2002)
42	<i>P. syriacus</i>	Prdejci	Macedonia	7	71.57	6	70.23	-1.02	(Ugurtaş et al., 2002)
43	<i>P. syriacus</i>	Djavato	Macedonia	10	76.52	13	71.51	-1.07	(Ugurtaş et al., 2002)
44	<i>P. syriacus</i>	Ezerani	Macedonia	11	60.59	13	59.93	-1.01	(Ugurtaş et al., 2002)
45	<i>P. syriacus</i>	Kresna	Bulgaria	14	76.89	4	72.88	-1.06	(Ugurtaş et al., 2002)
46	<i>P. syriacus</i>	Durankulak	Bulgaria	17	67.82	13	69.57	1.03	(Ugurtaş et al., 2002)
47	<i>P. syriacus</i>	Primorsko	Bulgaria	14	65.62	4	62.91	-1.04	(Ugurtaş et al., 2002)
48	<i>P. syriacus</i>	Edirne	Turkey	21	68.63	5	69.37	1.01	(Ugurtaş et al., 2002)
49	<i>P. syriacus</i>	Izmir	Turkey	12	70.83	9	62.22	-1.13	(Ugurtaş et al., 2002)
50	<i>P. syriacus</i>	Seydisehir	Turkey	13	63.08	9	58.17	-1.08	(Ugurtaş et al., 2002)
51	<i>P. syriacus</i>	Tbilisi	Georgia	16	71.6	9	71.5	-1.00	(Delwig, 1928)
52	<i>P. syriacus</i>	Izmir	Turkey	12	70.8	9	62.2	-1.13	(Baoğlu and Zaloglu, 1964)
53	<i>P. syriacus</i>		Grecia	38	72.4	44	73.2	1.01	(Sofianidou, 1977)
54	<i>P. syriacus</i>	Utrine	Serbia	21	61.13	30	60.83	-1.00	(Rot-Nikčević et al., 2001)
55	<i>P. syriacus</i>		Macedonia and Bulgaria	19	75.81	11	70.51	-1.07	(Rot-Nikčević et al., 2001)
56	<i>P. syriacus</i>	Balta Albă	Romania	10	69.1	2	73.8	1.07	Own unpublished data
57	<i>P. syriacus</i>	Grindul Lupilor	Romania	38	76.5	40	74.4	-1.02	Present study