

INTERANNUAL VARIATION IN LIFE HISTORY TRAITS BETWEEN NEIGHBOURING POPULATIONS OF THE WIDESPREAD AMPHIBIAN *BUFO BUFO*Nataša TOMAŠEVIĆ¹, Dragana CVETKOVIĆ², Claude MIAUD³, Ivan ALEKSIĆ¹
& Jelka CRNOBRNJA-ISAILOVIĆ¹

RÉSUMÉ. — *Variation interannuelle des traits d'histoire de vie entre des populations voisines de l'amphibien à grande aire de répartition, Bufo bufo.*— Les variations intra- et interpopulationnelles des traits d'histoire de vie chez les amphibiens à grande aire de répartition font l'objet d'une attention soutenue. Pour de nombreux problèmes (e.g. les changements climatiques, variations clinales, conservation), cette information est nécessaire, non seulement à une large échelle géographique, mais aussi au niveau local et d'une année à une autre. Dans cette étude nous avons étudié durant trois années successives les variations de la taille corporelle, de l'âge et des traits d'histoire de vie, y compris la fécondité et la taille des œufs, dans deux populations voisines du Crapaud commun *Bufo bufo*, une espèce à grande aire de répartition. La taille corporelle des mâles varie significativement entre les populations mais pas d'une année à une autre. L'âge a été déterminé par squelettochronologie et les analyses ostéologiques révèlent une résorption endostéale complète de la première ligne d'arrêt de croissance chez 1,1 % des individus et une résorption partielle chez 77 % des individus. Les structures d'âge ne diffèrent pas entre les sexes, les populations, ni entre les années. Les femelles se reproduisent pour la première fois un an après les mâles dans les deux populations (4 vs 3 ans), mais le taux de survie des adultes ne diffère pas entre les sexes. La fécondité ne varie pas entre les populations ou les années, mais la taille des œufs change d'une année à une autre. La fécondité augmente avec la taille, mais pas avec l'âge des femelles. Cette absence de variation de la structure d'âge et des paramètres de la reproduction est importante pour la surveillance des populations locales qui peuvent être exposées à divers facteurs de perturbation potentiels. Ces résultats soulignent également l'importance d'analyser les variations temporelles des traits d'histoire de vie car les données d'une année peuvent ne pas représenter correctement les caractéristiques de la reproduction d'une population, cette question étant exacerbée par l'intérêt croissant sur l'impact possible des changements climatiques sur les populations d'amphibiens.

SUMMARY. — *Life-history variation within and among populations of widespread amphibian species has attracted much attention so far. In relation to a variety of problems (e.g. climate change, large-scale clines, conservation), this information is needed not only on broad geographic scale, but also at local level and from year to year. In this study, we investigated variation in body size and life history traits, including age, fecundity and egg size, in two neighbouring populations of a widespread amphibian, the Common Toad Bufo bufo, during a 3-year period. Body size varied significantly between populations in males only, but not between years. Age was assessed by skeletochronology, and osteometrical analyses revealed a complete endosteal resorption of the first line of arrested growth in 1.1 % of the individuals and a partial resorption in 77 % of the individuals. Age structures did not differ significantly between sexes, populations, nor between years. Age at first reproduction was one year later in females than in males in both populations (4 vs 3 years), but adult survival rates did not differ between sexes. Fecundity did not differ between populations or years, but egg size varied between years. Fecundity increased with size, but not with age of females. This lack of significant interpopulation variation in age structure and reproductive traits is important for monitoring local populations which can be exposed to various potential disturbance factors. On the other hand, these results*

¹ Department of Evolutionary Biology, Institute for Biological Research, Blvd. Despota Stefana 142, 11060 Belgrade, Serbia

² Institute of Zoology, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia

³ Corresponding author. Université de Savoie, UMR CNRS 5553 Laboratoire d'Ecologie Alpine. F-73376 Le Bourget du Lac. E-mail: claudie.miaud@univ-savoie.fr

stress the importance of analysing temporal variation in life history traits because one-year data may not represent properly the reproductive features of a population, and this issue is becoming more so important with the growing interest in climate changes and its possible effects on amphibian populations.

Investigations of life-history variation within and among populations of various amphibian species have attracted much attention, especially with growing interest in problems of amphibian decline and conservation (e.g. Hitchings & Beebee, 1998; Eaton *et al.*, 2005; Reading, 2007). The importance of scale in ecological studies in general is well-known (e.g. Schneider, 2001); estimating e.g. species vulnerability requires information about life history variation not only on broad geographic scale, but about spatial and temporal variation patterns at local level as well. The range of intraspecific variation in life history traits can be very broad (Roff, 1992) and should be thoroughly studied to understand species conservation requirements, leading to development of appropriate conservation plans (Eaton *et al.*, 2005).

Widely distributed anuran species can be of particular interest since, over their wide geographic ranges, variation in climatic conditions, food availability and quality, etc., may maintain large amount of phenotypic variation in life history traits (review in Miaud *et al.*, 1999). They can be useful indicators of environmental changes such as habitat destruction, pollution, urbanization, etc. (Hitchings & Beebee, 1998; Lebboroni *et al.*, 2006). However, studies on local scale are necessary, since the severity of population change cannot be estimated without data describing its dynamics before disturbance. Geographic patterns of body size variation are of special interest in the context of current debate about Bergmann's rule in ectotherms and the question whether amphibians follow this rule (Ashton, 2002; Laugen *et al.*, 2005; Adams & Church, 2008). It has been concluded that, to answer this question, more case studies based on high-resolution data are needed (Laugen *et al.*, 2005). The Common Toad *Bufo bufo* is an anuran species with wide geographic and altitudinal distribution – from northwestern Africa, over entire Europe (except Ireland and some Mediterranean islands) east to the District of Irkutsk in Russia (Borkin & Veith, 1997; Frost, 2004). While demographic life history traits in populations from western part of European range are well studied (Gittins *et al.*, 1984; Hemeelaar, 1988; Reading, 1991; Kuhn, 1994; Grossenbacher, 2002), data from southern part of the continent are fragmentary (e.g. Cvetković *et al.*, 2003, 2005), and more surveys of temporal variation in these traits (Grossenbacher, 2002; Reading, 2003, 2007) are still needed. Brede & Beebee (2004) stressed the need for studies of life history variation in neighbouring local populations of this species, suggesting the existence of greater isolation and hence higher differentiation in this species compared to syntopic Common Frog *Rana temporaria*.

The aims of this study were: (1) to assess sex-specific variation in body size, age, potential reproductive lifespan and survival, (2) to assess variation in female reproductive traits (fecundity and egg size), and (3) to investigate temporal variation patterns of these traits in two neighbouring local populations of a widespread amphibian, the Common Toad *B. bufo*.

MATERIAL AND METHODS

STUDY SITE AND SAMPLING PROCEDURE

The study sites were: small lake Trešnja (44° 36' 26.6" N, 20° 34' 14" E, altitude 222 m a.s.l.) and the pond near Zuce village (44° 40' 55.9" N, 20° 33' 7.4" E, altitude 240 m), both situated in the agricultural area near Belgrade (Serbia) and surrounded with remnants of deciduous forests. Other amphibian species were also breeding there: *Pelophylax dalmatina*, *P. esculenta* complex, *Hyla arborea*, *Salamandra salamandra*, *Triturus vulgaris* and *T. cristatus* superspecies.

Adult toads were collected by hand around the breeding sites in the three consecutive years (2001 – 2003, sample sizes given in Table I). The impact of the sampling was expected to be small, considering population sizes (minimum estimates of adult census population size were 1396 and 1948 for Trešnja and Zuce, respectively; J. Crnobrnja-Isailović, unpublished data). Sampled adults were sexed by the external characteristics and the presence of gonads and were kept in Department of Evolutionary Biology of Institute for Biological Research in Belgrade for further studies.

BODY SIZE AND SKELETOCHRONOLOGICAL AGE ESTIMATION

Adult body length was measured from snout to vent (SVL) to the nearest 0.1 mm using dial caliper. Sexual size dimorphism was quantified according to Lovich & Gibbons (1992). Skeletochronological procedure for age estimation followed those previously described (Miaud, 1992; Cvetković *et al.*, 2005). Lines of arrested growth (LAGs) were counted and independently checked by at least three of the authors, to avoid possible under- or overestimation of age. Skeletochronology is accepted as a powerful tool for demographic studies in amphibians, but caution is needed with respect to actual number of LAGs. One of the factors affecting accuracy of age estimates is endosteal resorption. In *B. bufo*, it is a common phenomenon, which could lead to complete removal of the first and sometimes even the second LAG (Hemelaar, 1985; Frétey & Le Garff, 1996). The degree of resorption in our sample was estimated by osteometrical analysis (Sagor *et al.*, 1998). Cross-sections were recorded by digital camera Cannon S45 and processed with the image analysis software Image Tool (v. 3.0, UTHSC SA). The longest and shortest perpendicular axis of each LAG was measured to the nearest μm , and the square root of their product was considered as the average diameter of the LAG. Cross-sections where the diameter of the innermost visible LAG exceeded mean value for the group by more than two standard deviations were interpreted as the cases of complete LAG 1 resorption.

Adult survival rate was calculated from the age structure according to formula (Krebs, 1999):

$$S = T / (\Sigma N + T - 1)$$

where S is average survival rate, T is the sum of coded ages times their frequencies and ΣN is number of individuals from age classes x to $x + 1$ (details in Miaud *et al.*, 1999). The respective confidence intervals (C.I.) were calculated according to Zar (1984) and Kalezić *et al.* (1996) as:

$$\text{C.I.} = \pm 1.96 [S(1 - S) / (n - 1)]^{1/2}$$

Adult survival rates were used to calculate adult life expectancy (i.e. the expected average longevity of individuals that reached maturity, which is different from maximum recorded age) according to formula (Seber, 1973):

$$\text{ESP} = 0.5 + 1 / (1 - S)$$

The potential reproductive lifespan (PRLS) was defined as the difference between maximal and minimal recorded age of breeding adults (Leskovar *et al.*, 2006).

FEMALE REPRODUCTIVE TRAITS

Fecundity (egg number) and egg size (i.e. clutch parameters) were determined for a sample of 66 females – 33 from each population. Image analysis software Image Tool (v. 3.0, UTHSC SA) was used to assess total number of eggs, as well as egg diameter. Egg size was measured in a randomly chosen sample of 60 eggs from each female and the mean value was used in further analyses.

STATISTICAL ANALYSES

Age structures were analysed using Kolmogorov-Smirnov two-sample test and Kruskal-Wallis test. The average age is also given as the median of age distribution. Data distributions were tested and transformations (logarithmic) applied where necessary. Variation in body size and female reproductive traits were analysed using two-factor ANOVA with the locality and year of sampling as factors. Multiple comparison test (Tukeys HSD) was applied to temporal variation in egg size.

RESULTS

BODY SIZE

Summary of descriptive statistics for body size is presented in Table I. *Bufo bufo* is known for the pronounced sexual dimorphism in size (in this study also, males were significantly smaller than females: t-test, $p < 0.0001$ in all samples), so the sexes were analysed separately. Analysis of body size variation between localities and years showed that only the effect of locality on male body size was significant (Tab. I).

OSTEOMETRICAL ANALYSIS AND AGE STRUCTURE

Skeletochronological analysis was performed on phalangeal cross sections of 187 individuals. LAGs were clearly distinguishable from the rings of periosteal bone deposited during the activity period. Individual variation in the degree of resorption was pronounced. Figure 1 shows the distribution of diameters of the first and the second visible LAG in phalangeal cross-sections. Complete resorption of the first LAG was observed in two males only (1.1 %), while partial resorption was found in 77 % of the individuals. In 20 % of the individuals partial resorption of the second LAG was found as well. We thus concluded that in the analysed

TABLE I

Mean body length (SVL), standard errors and range (minimal and maximal values), of male and female *B. bufo* from Trešnja and Zuce (Measurements in mm; n = number of individuals (Trešnja 2003 – no males due to accidental loss). Two-factor ANOVA, i.e. the effects of locality, year of sampling and interaction (*one year less in males)

locality	year	males			females		
		n	mean \pm S.E.	range	n	mean \pm S.E.	range
TREŠNJA	2001	47	67.4 \pm 0.68	55.9-80.9	23	92.0 \pm 1.64	78.5-112.8
	2002	44	67.0 \pm 0.61	59.7-76.1	20	90.7 \pm 1.55	72.2-98.5
	2003	/	/	/	14	96.9 \pm 1.50	90.4-107.9
ZUCE	2001	7	73.2 \pm 2.79	63.1-84.4	8	97.0 \pm 1.97	89.9-104.5
	2002	9	68.2 \pm 1.49	60.4-72.8	10	95.3 \pm 2.84	84.4-111.9
	2003	25	70.4 \pm 0.70	63.9-77.6	33	96.5 \pm 1.37	78.3-110.1
Two-factor ANOVA		males			females		
		F	p		F	p	
locality		6.125	0.015		3.613	0.060	
year*		3.702	0.057		2.058	0.133	
locality x year		2.622	0.108		1.377	0.257	

In bold : statistically significant values

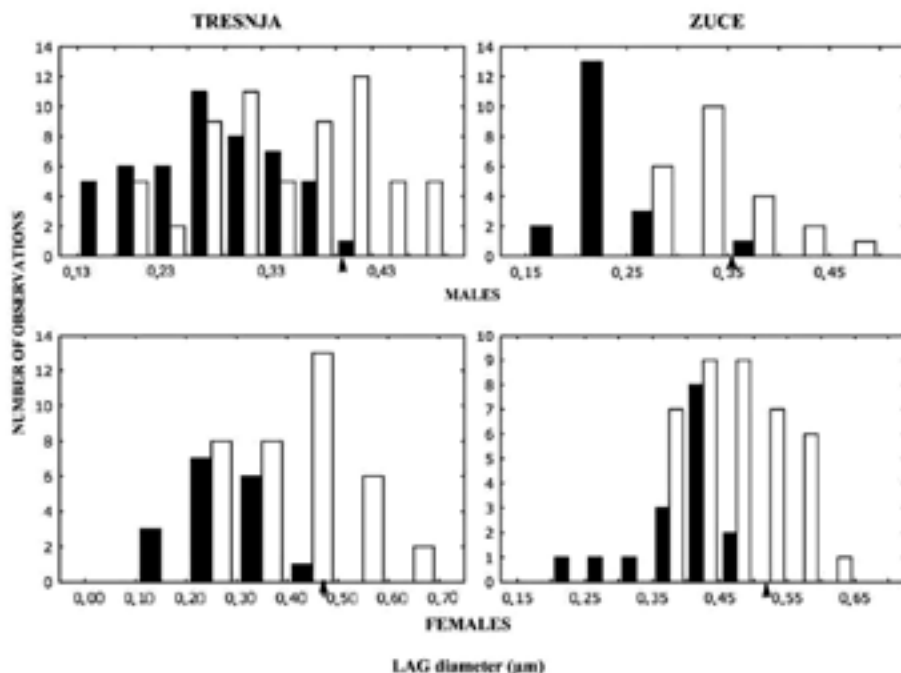


Figure 1. — Diameter distributions of the first (innermost) and second visible Line of Arrested Growth (LAG) in phalangeal cross-section of *B. bufo* from Trešnja and Zuce populations (black bar = first LAG, white bar = second LAG). Arrows represent mean diameter of the first LAG + 2SD; cases beyond this limit represent the cases of complete LAG # 1 resorption.

populations endosteal resorption was pronounced, but not to the extent that would affect the accuracy of age estimates.

Age distributions (Fig. 2) show that age at first reproduction was one year later in females than in males in both populations. Age structures did not differ significantly between sexes, populations, nor between years (Kolmogorov-Smirnov and Kruskal-Wallis test, $p > 0.10$ in all cases).

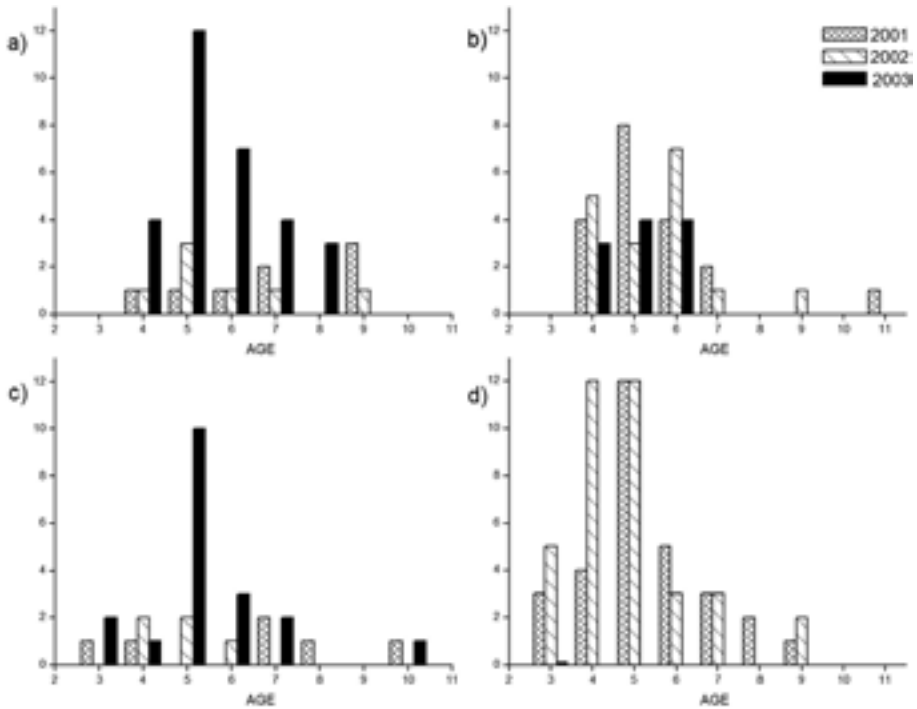


Figure 2. — Age distributions of *B. bufo* from Trešnja and Zuce populations for the years 2001-2003: a = Zuce females, b = Trešnja females, c = Zuce males, d = Trešnja males.

The overview of body size, age and survival features (Tab. II) is given for pooled data sets. The youngest breeding adults belonged to age classes 3 (males) and 4 (females). Minimal body size in these classes was 60.1 mm and 67.2 mm (males, Trešnja and Zuce, respectively), 75.2 mm and 92.5 mm (females, Trešnja and Zuce, respectively). However, minimal adult body sizes for the total sample (Tab. II) were recorded in older individuals, confirming the poor value of size as age predictor in *B. bufo*.

TABLE II

Variability of analysed life history traits in male and female B. bufo from Trešnja and Zuce populations

	Trešnja		Zuce	
	males	females	males	females
SIZEM	65.27 ± 1.72	88.75 ± 2.39	68.89 ± 0.87	96.85 ± 2.75
AGEM	3	4	3	4
MED	5	5	5	6
PRLS	6	7	7	5
AGEVAR	2	1	2	1
% EBR	12.31 %	25.53 %	10.0 %	13.33 %
S (C.I.)	0.67 (0.12)	0.59 (0.14)	0.75 (0.16)	0.67 (0.14)
ESP	3.5	3	4.5	3.5

SIZEM = size at maturity; AGEM = age at maturity; MED = median age; PRLS = potential reproductive lifespan; AGEVAR = age variation in first breeders; % EBR = percent of early breeders; S = survival rate; C.I. = confidence interval; ESP = adult life expectancy; size in mm; age measurements in years

The early breeding adults accounted for 10-13 % of all adults, the exception being Trešnja females (25.5 %, Tab. II). Age variation in first breeders, calculated as difference between the age of the cohort forming the maximum of unimodal age distribution and the minimum age of reproductive individuals (*sensu* Leskovar *et al.*, 2006), did not differ between populations – it was 2 years for males and 1 year for females.

Adult survival rates, estimated from age structure, were lower in females than in males of both populations. However, the differences were not considered significant, since the respective C.I.'s broadly overlapped.

Figure 3 shows the variation in sexual size dimorphism (i.e. index SDI) for age classes 4 to 7, where sample size allowed its estimation. No consistent trend of change in SDI was found, contrary to previous finding in *R. temporaria* (Miaud *et al.*, 1999) that SDI showed the tendency to decrease with age.

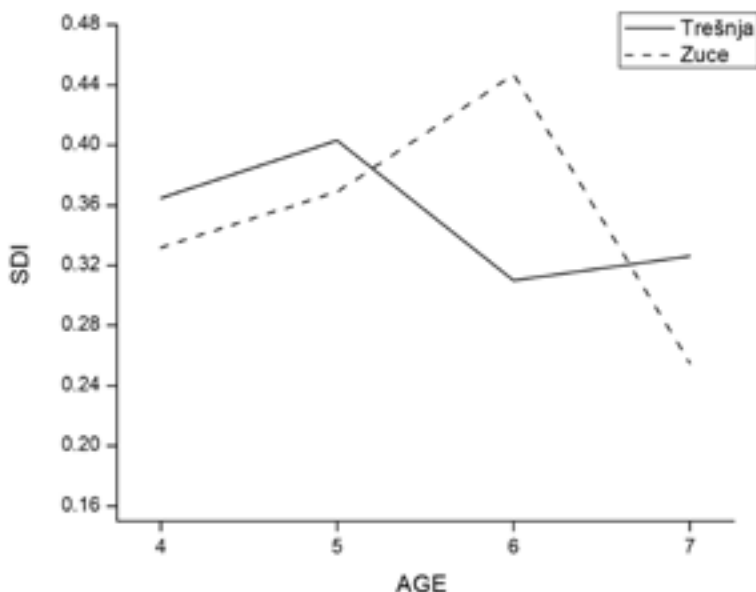


Figure 3. — Variation of sexual size dimorphism index (SDI) in age classes 4-7 of *B. bufo* from Trešnja and Zuce populations.

FEMALE REPRODUCTIVE TRAITS

Fecundity (number of eggs per female) and egg size variation data are given in Table III. In Trešnja population, minimal number of eggs per female was recorded in 2001, and maximal in 2003; in Zuce population, both minimal and maximal values were recorded in 2003. Coefficients of variation for egg size ranged from 0.06 to 0.18 in Trešnja, and from 0.08 to 0.16 in Zuce.

Variation in fecundity and egg size was analysed using two-factor ANOVA (factors population and year of sampling), with body size and age as covariates. Fecundity did not differ significantly between populations, nor between years (Tab. III). Mean egg size significantly varied between years (two-factor ANOVA, $F = 7.69$, $p < 0.01$) while differences between populations and interactions (locality x year) were not significant. In both populations, mean egg diameter was significantly larger in 2003 sample, compared to other two samples (multiple comparison test – Tukeys HSD test, $p < 0.01$).

TABLE III

Fecundity (number of eggs per female) and egg size (in mm) of female B. bufo from Trešnja and Zuce populations. Two-factor ANOVA, i.e. the effects of locality, year of sampling and interaction

		fecundity		egg size	
locality	year	mean \pm S.E.	range	mean \pm S.E.	range
TREŠNJA	2001	5963.8 \pm 530.8	2118 – 8483	2.23 \pm 0.05	2.09 – 2.75
	2002	8864.0 \pm 932.4	5476 – 11321	2.67 \pm 0.06	2.53 – 2.87
	2003	8005.8 \pm 610.9	5366 – 12444	2.90 \pm 0.14	2.27 – 4.47
ZUCE	2001	7169.2 \pm 762.2	4731 – 10486	2.43 \pm 0.14	2.04 – 3.27
	2002	6298.0 \pm 1324.4	4685 – 8924	2.42 \pm 0.11	2.31 – 2.64
	2003	7114.5 \pm 674.5	3024 – 15050	3.07 \pm 0.05	2.27 – 3.46
Two-factor ANOVA		fecundity		egg size	
		F	p	F	p
locality		1.10	0.30	0.21	0.65
year		0.26	0.78	7.69	0.009
locality x year		2.99	0.06	0.55	0.46

In bold : statistically significant values

Fecundity was significantly correlated with body size in both populations (Trešnja: $R_s = 0.48$, $p = 0.008$; Zuce: $R_s = 0.60$, $p < 0.001$), contrary to egg size. Fecundity and egg size did not vary with the age of females (fecundity, $R_s = -0.39$, $p = 0.07$ and $R_s = -0.19$, $p = 0.27$ and egg size, $R_s = 0.005$, $p = 0.99$, $R_s = 0.38$, $p = 0.09$ in Trešnja and Zuce respectively). The correlation between fecundity and mean egg diameter was negative, but not significant (Trešnja: $R_s = -0.25$, $p = 0.30$; Zuce: $R_s = -0.34$, $p = 0.06$).

DISCUSSION

Growing interest in widely distributed anuran species has resulted in a body of studies dealing with various aspects of their life history and genetic characteristics, habitat quality, etc. (Scribner *et al.*, 1994, 2001; Sztatecsny & Schabetsberger, 2005; Brede & Beebee, 2006; Kutrup *et al.*, 2006; Reading, 2007). Like other widespread anurans, *B. bufo* can be used as indicator of environmental changes on a large scale, provided that patterns of intraspecific variation in life history traits are known throughout its range.

Considerable variation in adult body size, age and size at maturity, documented in various amphibians (Hemelaar, 1988; Ryser, 1996; Miaud *et al.*, 1999; Morrison *et al.*, 2004), can arise from several factors, including genetic differences and differences in environmental conditions such as duration of the activity period, food availability and quality, climatic conditions, etc. Studies concerning neighbouring populations often focused on local ecological conditions as the main explanation for the observed interpopulation differences (Augert & Joly, 1993; Miaud *et al.*, 2001).

In Trešnja and Zuce populations body size variation was considerable, but the differences between populations were significant only for males, and interannual variation was not significant. Age distributions in analysed populations did not differ significantly between sexes, populations, nor between years. Based on life history theory, we could expect that, since sexes differ in reproductive investment, their age structures also may vary (Charlesworth, 1980). In addition, age differences in favour of males are expected, that are associated with higher mortality of females during reproductive period, especially with regard to the fierce mate competition and the sex-ratio in this species. In *B. bufo* sex ratio is often considerably male biased, with reported ratios from 1.9:1 to 8.3:1 (review in Brede & Beebee, 2006). Our observations confirm this bias; in Trešnja and Zuce populations the operational sex ratio varied from 2:1 to

as high as 9:1 (J. Crnobrnja-Isailović *et al.* unpublished data). However, we did not find significant intersexual differences in age structure, nor the expected (Frétey *et al.*, 2004) sex-specific differences in survival; the most prominent feature was different age at first reproduction.

Females reproducing one year later than males were often reported in *B. bufo* (Gittins *et al.*, 1982; Hemelaar, 1988; Frétey & Le Garff, 1996; Schabetsberger *et al.*, 2000; Grossenbacher, 2002). Hemelaar (1988) found that maturation may occur after minimum number of growing years is experienced, but not before a given minimum size (dependent on genotype, climatic and other environmental factors and their interactions) is reached. Females take longer time to reach this minimum size and are older than males when they breed for the first time, but delayed maturation allows them a prolonged period of rapid growth as juvenile without investment in costly (e.g. oocytes) reproductive traits.

Understanding the age trade-offs of reproduction (Roff, 1992), i.e. the costs and benefits of maturing at various ages and sizes, is one of the central aims of life history analysis in amphibians. The benefits of maturing early include early reproduction (but at smaller body size), shorter generation length and higher rate of population increase (Begon *et al.*, 1996). Since in amphibians growth rates decrease after maturation (Hemelaar, 1988), the benefits of delayed reproduction are associated with prolonged growth and, consequently, attainment of larger size. Larger body size is typically associated with increased fecundity (Gibbons & McCarthy, 1986), and often with production of larger offspring and, in males, success at obtaining a mate (Berven, 1982; Begon *et al.*, 1996; Castellano *et al.*, 2004). In Trešnja and Zuce, correlation between female body length and fecundity was significantly positive, which points to selective pressure for large body in females (fecundity selection is considered one of the major determinants of the direction and magnitude of sexual size dimorphism in Common Toad). The same results were reported for numerous European Common Toad populations (e.g. Reading, 1986; Høglund & Robertson, 1987; Kuhn, 1994), though some studies (Gittins *et al.*, 1984; Van Gelder, 1995) revealed no significant correlation between these two parameters. Fecundity did not differ significantly between populations or years in our study. Mean egg size varied significantly among years (larger in 2003), but not between populations. Within-population variation in egg size among amphibians has been well documented (e.g. Cummins, 1986); the levels of variation observed in the studied populations are in accordance with those previously reported for this species (Kuhn, 1994). With regard to the expected trade-off between egg size and number, we found negative, but nonsignificant correlation between these variables in the studied populations. Mean egg size was not significantly correlated with egg number in other studies on *B. bufo* (Gittins *et al.*, 1984; Høglund & Robertson, 1987) as well. The same results were obtained also for *Bufo calamita* (Tejedo, 1992), *B. viridis* (Castellano *et al.*, 2004), and for all 15 anuran species surveyed in Dziminski & Alford (2005).

Egg size, which influences the survival of offspring and the larval success (and thus has fitness consequences, Dziminski & Alford, 2005; Thumm & Mahony, 2005) is affected by a number of factors, internal as well as external. One of these is the available amount of energy, which is associated with the length of the activity period (Morrison & Hero, 2003). Kuhn (1994) found that egg size increased with body length and somatic condition. Contrary to this, we found non-significant relationship between female body length and egg size. Similar results were reported previously for *B. bufo* (e.g. Gittins *et al.*, 1984), but in *B. calamita* (Tejedo, 1992) and *B. viridis* (Castellano *et al.*, 2004) larger individuals produced larger clutches, both in the number and size of eggs. In addition, studies on other ectotherms showed that body size had a highly important role in shaping the relationship between clutch size and egg size (Kolm *et al.*, 2006). On the other hand, significant interannual variation in egg size in this study points to variation in somatic condition, which is under the influence of extrinsic (climatic) factors. Factors such as temperature and humidity can directly affect the availability of food, which affects the fat stores and, consequently, the egg size. Our study of variation in body condition index (BCI) before spawning (Tomašević *et al.*, 2007) has shown that winter temperatures had strong impact on somatic condition of toads emerging from hibernation and also, that mean egg diameter was positively correlated with BCI.

To conclude, we have here provided data on local patterns of variation in body size and demographic life history traits in a widespread anuran species, which could contribute to the «high resolution» analysis of large-scale clines, for example in the context of the current debate about Bergmann's rule in amphibians. Data on geographic variation in traits other than age and body size are particularly scarce. For example, it appears that, even in a well studied species like *B. bufo*, there is insufficient data to analyse fecundity variation along geographic gradients. In the context of closely situated breeding units, we found a relatively low level of phenotypic differentiation in various life history characteristics. The present lack of significant interpopulation variation in age structure and female reproductive traits represents valuable information for monitoring on the local scale in relation to various disturbance factors. On the other hand, this study stresses the importance of analysing temporal variation in life history traits which can exhibit significant interannual variation, due to extrinsic factors. As a consequence, only long term studies can take into account this issue, and have to be promoted especially with the growing interest in climate change and its possible effects on amphibian populations.

ACKNOWLEDGEMENTS

This study was approved and supported by Ministry of Science and Nature Protection of Republic of Serbia (grant No.143040). The authors thank S. Mančić, S. Radosavljević, J. Jakovljević and N. Jeličić for technical assistance, D. Cogalniceanu, T. Frétey and reviewers for constructive comments.

REFERENCES

- ADAMS, D.C. & CHURCH, J.O. (2008). — Amphibians do not follow Bergmann's rule. *Evolution*, 62: 413-420.
- ASHTON, K.G. (2002). — Do amphibians follow Bergmann's rule? *Can. J. Zool./Rev. can. Zool.*, 80: 708-716.
- AUGERT, D. & JOLY, P. (1993). — Plasticity of age at maturity between two neighbouring populations of the common frog (*Rana temporaria* L.). *Can. J. Zool. /Rev. can. Zool.*, 71: 26-33.
- BEGON, M., HARPER, J.L. & TOWNSEND, C.R. (1996). — *Ecology: individuals, populations and communities*. 3rd ed. Blackwell Science, London.
- BERVEN, K.A. (1982). — The genetic basis of altitudinal variation in the wood frog, *Rana sylvatica*. a) An experimental analysis of life-history traits. *Evolution*, 36: 962-983.
- BORKIN, L.J. & VEITH, M. (1997). — *Bufo bufo*. Pp 118-119 In: J.P. Gasc, A. Cabela, J. Crnobrnja-Isailović, D. Dolmen, K. Grossenbacher, P. Haffner, J. Lescure, H. Martens, J.P. Martinez Rica, H. Maurin, M.E. Oliveira, T.S. Sofianidou, M. Veith & A. Zuiderwijk (eds). *Atlas of Amphibians and Reptiles in Europe*. Societas Europaea Herpetologica & Muséum National d'Histoire Naturelle (IEGB/SPN), Paris.
- BREDE, E.G. & BEEBEE, T.J. (2004). — Contrasting population structures in two sympatric anurans: implications for species conservation. *Heredity*, 92: 110-117.
- BREDE, E.G. & BEEBEE, T.J. (2006). — Large variations in the ratio of effective breeding and census population sizes between two species of pond-breeding anurans. *Biol. J. Linn. Soc.*, 89: 365-372.
- CASTELLANO, S., CUCCO, M. & GIACOMA, C. (2004). — Reproductive investment of female green toads (*Bufo viridis*). *Copeia*, 2004: 659-664.
- CHARLESWORTH, B. (1980). — *Evolution in age structured populations*. Cambridge University Press, Cambridge.
- CUMMINS, C.P. (1986). — Temporal and spatial variation in egg size and fecundity in *Rana temporaria*. *J. Animal Ecol.*, 55: 303-316.
- CVETKOVIĆ, D., ALEKSIĆ, I. & CRNOBRNJA-ISAILOVIĆ, J. (2003). — Reproductive traits in common toad *Bufo bufo* from the vicinity of Belgrade. *Arch. Biol. Sci. Belgrade*, 55: 25P-26P.
- CVETKOVIĆ, D., TOMAŠEVIĆ, N., ALEKSIĆ, I. & CRNOBRNJA-ISAILOVIĆ, J. (2005). — Assessment of age and intersexual size differences in *Bufo bufo*. *Arch. Biol. Sci. Belgrade*, 57: 157-162.
- DZIMINSKI, M.A. & ALFORD, R.A. (2005). — Patterns and fitness consequences of intraclutch variation in egg provisioning in tropical Australian frogs. *Oecologia*, 146: 98-109.
- EATON, B., PASZKOWSKI, C.A., KRISTENSEN, K. & HILTZ, M. (2005). — Life-history variation among populations of Canadian Toads in Alberta, Canada. *Can. J. Zool./Rev. can. Zool.*, 83: 1421-1430.
- FRÉTEY, T. & LE GARFF, B. (1996). — Skeletochronological study in *Bufo bufo* in Brittany. *CR Acad. Sci. III*, 319: 295-299.
- FRÉTEY, T., CAM, E., LE GARFF, B. & MONNAT, J.Y. (2004). — Adult survival and temporary emigration in the common toad. *Can. J. Zool./Rev. can. Zool.*, 82: 859-872.

- FROST, D.R. (2004). — *Amphibian species of the world: An online reference*. Version 3.0 <http://research.amnh.org/herpetology/amphibia/index.php>. American Museum of Natural History, New York.
- GIBBONS, M.M. & MCCARTHY, T.K. (1986). — The reproductive output of frogs *Rana temporaria* (L.) with particular reference to body size and age. *J. Zool.*, 209: 579-593.
- GITTINS, S.P., STEEDS, J.E. & WILLIAMS, R. (1982). — Population age structure of the common toad (*Bufo bufo*) at a lake in Mid-Wales determined from annual growth rings in the phalanges. *Brit. J. Herpet.*, 6: 249-252.
- GITTINS, S.P., KENNEDY, R.I. & WILLIAMS, R. (1984). — Fecundity of the common toad (*Bufo bufo*) at a lake in Mid-Wales. *Brit. J. Herpet.*, 6: 378-380.
- GROSSENBACHER, K. (2002). — First results of a 20-year study on common toad *Bufo bufo* in the Swiss Alps. *Biota*, 3: 43-48.
- HEMELAAR, A. (1985). — An improved method to estimate the number of year rings resorbed in phalanges of *Bufo bufo* (L.) and its application to populations from different latitudes and altitudes. *Amphib.-Reptilia*, 6: 323-341.
- HEMELAAR, A. (1988). — Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes. *J. Herpetol.*, 22: 369-388.
- HITCHINGS, S.P. & BEEBEE, T.J.C. (1998). — Loss of genetic diversity and fitness in Common Toad (*Bufo bufo*) populations isolated by inimical habitat. *J. Evol. Biol.*, 11: 269-283.
- HOGLUND, J. & ROBERTSON, J.G.M. (1987). — Random mating by size in a population of common toads (*Bufo bufo*). *Amphib.-Reptilia*, 8: 321-330.
- KALEZIĆ, M.L., CVETKOVIĆ, D., DJOROVIĆ, A. & DŽUKIĆ, G. (1996). — Alternative life-history pathways: paedomorphosis and adult fitness in European newts (*Triturus vulgaris* and *T. alpestris*). *J. Zool. Syst. Evol. Res.*, 34: 1-7.
- KOLM, N., GOODWIN, N.B., BALSHINE, S. & REYNOLDS, J.D. (2006). — Life history evolution in cichlids 2: directional evolution of the trade-off between egg number and egg size. *J. Evol. Biol.*, 19: 76-84.
- KREBS, C.J. (1999). — *Ecological methodology*. 2nd ed., Addison, Wesley, Longman, Menlo Park, California.
- KUHN, J. (1994). — Lebensgeschichte und Demographie von Erdkrotenweibchen *Bufo bufo bufo* (L.). *Zeitsch. für Feldherpet.*, 1: 3-87.
- KUTRUP, B., YILMAZ, N., CANAKCI, S., BELDUZ, A.O. & DOGLIO, S. (2006). — Intraspecific variation of *Bufo bufo*, based on 16S Ribosomal RNA sequences. *Amphib.-Reptilia*, 27: 268-273.
- LAUGEN, A.T., LAURILA, A., JONSSON, K.I., SODERMAN, F. & MERILA, J. (2005). — Do common frogs (*Rana temporaria*) follow Bergmann's rule? *Evol. Ecol. Research*, 7: 717-731.
- LEBBORONI, M., RICCHIARDINO, G., BELLAVITA, M. & CHELAZZI, G. (2006). — Potential use of anurans as indicators of biological quality in upstreams of central Italy. *Amphib.-Reptilia*, 27: 73-79.
- LESKOVAR, C., OROMI, N., SANUY, D. & SINSCH, U. (2006). — Demographic life history traits of reproductive natterjack toads (*Bufo calamita*) vary between northern and southern latitudes. *Amphib.-Reptilia*, 27: 365-375.
- LOVICH, J.E. & GIBBONS, J.W. (1992). — A review of techniques for quantifying sexual size dimorphism. *Growth Dev. Aging*, 56: 269-281.
- MIAUD, C. (1992). — La squelettechronologie chez les *Triturus* (Amphibiens, Urodèles) à partir d'une étude de *T. alpestris*, *T. helveticus* et *T. cristatus* du sud-est de la France. Pp 363-384 In: J.L. Baglinière et al. (eds), *Tissus durs et âge individuel des vertébrés*. Colloques et Séminaires ORSTOM-INRA, Bondy, France.
- 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. (Lond.)*, 249: 61-73.
- MIAUD, C., ANDREONE, F., RIBERON, A., MICHELIS, S. DE, CLIMA, V., CASTANET, J., FRANCILLON-VIEILLOT, H. & GUYETANT, R. (2001). — Variations in age, size at maturity and gestation duration among two neighbouring populations of the Alpine salamander *Salamandra lanzai*. *J. Zool. (Lond.)*, 254: 251-260.
- MORRISON, C. & HERO, J.M. (2003). — Geographic variation in life history characteristics of amphibians: a review. *J. Animal Ecol.*, 72: 270-279.
- MORRISON, C., HERO, J.M. & BROWNING, J. (2004). — Altitudinal variation in the age at maturity, longevity and reproductive lifespan of anurans in subtropical Queensland. *Herpetologica*, 60: 34-44.
- READING, C.J. (1986). — Egg production in the common toad, *Bufo bufo*. *J. Zool.*, 208: 99-107.
- READING, C.J. (1991). — The relationship between body length, age and sexual maturity in the common toad *Bufo bufo*. *Holarct. Ecol.*, 14: 245-249.
- READING, C.J. (2003). — The effects of variation in climatic temperature (1980-2001) on breeding activity and tadpole stage duration in the common toad, *Bufo bufo*. *Science of the Total Environment*, 310: 231-236.
- READING, C.J. (2007). — Linking global warming to amphibian declines through its effects on female body condition and survivorship. *Oecologia*, 15: 125-131.
- ROFF, D. (1992). — *The evolution of the life histories: theory and analysis*. Chapman and Hall, New York.
- RYSER, J. (1996). — Comparative life histories of low- and high-elevation population of the common frog *Rana temporaria*. *Amphib.-Reptilia*, 17: 183-195.
- SAGOR, E.S., OUELLET, M., BARTEN, E. & GREEN, D.M. (1998). — Skeletochronology and geographic variation in age structure in the wood frog, *Rana sylvatica*. *J. Herpetol.*, 32: 469-474.

- SCHABETSBERGER, R., LANGER, H., JERSABEK, C.D. & GOLDSCHMID, A. (2000). — On age structure and longevity in two populations of *Bufo bufo* (Linnaeus, 1758) at high altitude breeding sites in Austria. *Herpetozoa*, 13: 187-191.
- SCHNEIDER, D.C. (2001). — The rise of the concept of scale in ecology. *BioScience*, 51: 545-552.
- SCRIBNER, K.T., ARNTZEN, J.W. & BURKE, T. (1994). — Comparative analysis of intra- and interpopulation genetic diversity in *Bufo bufo*, using allozyme, single-locus microsatellite, minisatellite, and multilocus minisatellite data. *Mol. Biol. Evol.*, 11: 737-748.
- SCRIBNER, K.T., ARNTZEN, J.W., CRUDDACE, N., OLDHAM, R.S. & BURKE, T. (2001). — Environmental correlates of toad abundance and population genetic diversity. *Biol. Conserv.*, 98: 201-210.
- SEBER, G.A. (1973). — *The estimation of animal abundance and related parameters*. London, Griffin.
- SZTATECSNY, M. & SCHABETSBERGER, R. (2005). — Into thin air: vertical migration, body condition, and quality of terrestrial habitats of alpine common toads, *Bufo bufo*. *Can. J. Zool./Rev. can. Zool.*, 83: 788-796.
- TEJEDO, M. (1992). — Absence of the trade-off between the size and number of offspring in the natterjack toad (*Bufo calamita*). *Oecologia*, 90: 294-296.
- THUMM, K. & MAHONY, M. (2005). — Is variable egg size the proximate cause of diversified bet-hedging in the hatching dynamics of the red-crowned toadlet (*Pseudophryne australis*) (Anura: Myobatrachidae)? *Herpetologica*, 61: 9-19.
- TOMAŠEVIĆ, N., CVETKOVIĆ, D., ALEKSIĆ, I. & CRNOBRNJA-ISAILOVIĆ, J. (2007). — The effect of climatic conditions on post-hibernation body condition and reproductive traits of *Bufo bufo* females. *Arch. Biol. Sci. Belgrade*, 59: 51P-52P.
- VAN GELDER, J.J. (1995). — Reproductive effort in *Bufo bufo*. Pp 176-179 In: G. Llorente, A. Montori, X. Santos & M.A. Carretero (eds). *Scientia Herpetologica*. Barcelona,.
- ZAR, J.H. (1984). — *Biostatistical analysis*. 2nd ed., Prentice-Hall, New York.

