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MORPHOLOGICAL VARIATION AND POPULATION STRUCTURE OF THE NATTERJACK TOAD, *EPIDALEA CALAMITA*, IN NORTHERN PART OF THE RANGE IN BELARUS

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In the article the results of analysis the morphological variability, sex and size-age structure of the Natterjack toad *Epidalea calamita* population in Belarus are presented. The studied population differs by larger body size of the toads than indicated in the description for the whole eastern part of the range (Kuzmin, 1999). Statistically significant sex distinctions for some of the analyzed morphological parameters defined. The sex ratio during the active season varies considerably; because of difference the reproductive cycles and activity of males and females. The real sex ratio in the population is more balanced, with a slight predominance of males (1,1:1). In accordance with body length (L) the population of the Natterjack toad in Belarus is divided into 3 distinct size-age groups: juveniles, immature specimens under the age of 2 years, and adults older than 2 years. The lifespan of *E. calamita* in Belarus usually does not exceed 6 years; occasionally there were toads, whose age, according to the results of tagging, was estimated at 7-9 years. Medium-sized specimens with body length in the range 56-60,9 mm compose main group of the population.

Key words: Natterjack toad, morphological variation, structure, population, Belarus.

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INTRODUCTION

The Natterjack toad (*Epidalea calamita* Laurenti, 1768) is one of the most rare and poorly known species of amphibians of Eastern Europe (Fig. 1). In all countries this region, in Poland, Belarus, Lithuania, Latvia, Estonia, Ukraine and Russia *E. calamita* is rare, sporadic, threatened species included in the national red lists (Juszczyk 1974, Pikulik 1985, Gruodis et al. 1986, Gruodis et al. 1987, Zabroda 1989, Ernits 1990, Kuzmin 1999, Ananjeva et al. 2000, Drobenkov et al. 2005, Pupins & Pupina 2011, Čeirāns et al. 2015).

The current range of the Natterjack toad in Belarus covers only western and central parts of the country (Pikulik 1985, Drobenkov et al. 2005, Krasnaia kniga Respubliki Belarus 2015). The boundary of the geographical distribution

this species in the country is presently stretches near the line of the cities Glubokoe – Lepel – Bobruisk – Mozyr (Pikulik 1985, Drobenkov et al. 2005). Morphological variability and population patterns of the Natterjack toad in the eastern part of the range, to date, studied only in the common features. Despite the wide range, covering much of Europe, the Natterjack toad is monotypic species, characterized by low variability. For Belarus in the literature there are fragmentary data on morphological variability of this species, based on the small sample (Pikulik 1985, Bakcharev 1986, Gruodis et al. 1986, Bakharev et al. 1995, Novitsky 2001).

The relevance of the Natterjack toad study in Eastern Europe is associated with a number of questions connected necessity of morphological description the regional populations, interest in issues intraspecific differentiation this species and its morpho-physiological adaptations to extreme conditions of environment in the northern part of the range in cold climate zone.

This work therefore aims to analyze the morphological variation and population structure of the Natterjack toad in Belarus.

MATERIAL AND METHODS

Materials on morphological variation and sex and size-age structure of the Natterjack toad population were collected in 1991-2015 in Belarus in the area of the current distribution the species. The study was carried out in the Stolbtsy, Ivatsevichy, Gantsevichy, Malorita, Pinsk, Minsk and Grodno districts. Field studies conducted in the aquatic and terrestrial habitats of the Natterjack toad from April to September each year.

In the study area characteristic terrestrial habitats of *E. calamita* presented by transformed open biotopes including fields, meadows, marshes reclaimed, island pine and mixed forests (Drobenkov 2015). Often the Natterjack toad is found at sites of the urban and recreational landscapes – in villages, along roads, in suburban settlements, around lakes and reservoirs. Toads breed in small shallow artificial ponds, water-filled mined-out open pits, small drainage channels and temporary rainwater puddles.

Morphological description of the toads was conducted according to conventional schemes (Terentjev 1950, Bannikov et al. 1977, Pisanets 1989), with some original additions

Fig. 1. Natterjack toad *Epidalea calamita* in Belarus.

Table 1. Variation the morphometrical characteristics in the Natterjack toad *Epidalea calamita* population of Belarus

Morphometrical parameter	sex	min-max	X±*m*	CV	t, *p*
L, body length, mm	♂♂	49,8-80,0	62,1±0,5	9,41	2,14, p>0,95
L.cap, length of head, mm	♀♀ ♂♂ ♀♀	49,2-74,3 15,0-17,9 14,9-17,5	62,4±1,22 16,6±0,72 16,3±0,83	9,77 9,66 9,11	2,01, p>0,95
Lt.cap, head width, mm	♂♂	17,8-26,2	20,8±0,24	9,03	1,51, p<0,05
	♀♀	16,7-25,1	20,0±0,44	10,55	
D.n.o, distance from nostril to eye, mm	♂♂ ♀♀	3,0-5,6 3,4-5,1	4,3±0,05 4,1±0,15	12,24 14,23	1,19, p<0,05
Sp.n, distance between the nostrils, mm	♂♂ ♀♀	3,1-5,5 2,9-5,0	4,2±0,05 4,3±0,14	23,27 12,77	2,61, p>0,99
L.o, length of eye, mm	♂♂	4,3-9,0	6,1±0,13	18,86	4,04, p>0,99
	♀♀	5,0-9,0	6,8±0,33	18,85	
L.o.tym, distance between eye and tympanic membrane, mm	♂♂ ♀♀	0,5-3,1 1,3-2,9	1,9±0,06 2,2±0,14	25,72 23,03	1,92, p<0,05
Lt.tym, vertical diameter of tympanic membrane, mm	♂♂ ♀♀	2,8-5,7 2,1-5,5	4,2±0,07 3,7-0,19	15,68 26,77	3,01, p>0,99
L.tym, horizontal diameter of tympanic membrane, mm	♂♂ ♀♀	2,5-6,2 2,4-5,1	4,1±0,08 4,0±0,19	17,89 18,64	2,31, p>0,05
F, length of femur, mm	♂♂	17,0-30,5	23,1±0,25	11,28	2,91, p>0,99
	♀♀	15,6-33,3	21,1±0,71	16,75	
T, tibia length, mm	♂♂	17,9-27,0	22,2±0,21	8,89	3,5, p>0,99
	♀♀	14,9-24,3	20,2±0,47	11,60	
D.p, length of first digit of hind limb, mm	♂♂ ♀♀	1,9-5,8 2,4-4,2	3,2±0,08 3,3±0,19	20,03 20,45	1,83, p<0,05
C.int, length of inner calcaneal tuber, mm	♂♂ ♀♀	1,8-4,4 2,9-4,5	3,2±0,07 3,4±0,15	19,13 16,18	2,64, p<0,05
L.par, length parotid, mm	♂♂	5,7-14,0	10,2±0,18	16,10	2,14, p<0,05
	♀♀	6,9-14,5	10,1±0,34	16,75	
Lt.par, width parotid, mm	♂♂	4,1-12,3	6,4±0,17	24,63	3,67, p>0,99
	♀♀	4,0-7,7	5,5±0,19	17,44	
m, body mass, g	♂♂	12,1-35,1	20,9±0,52	25,80	3,21, p>0,95
	♀♀	13,0-38,1	25,0±0,59	29,72	

Table 2. Geographic variation in body length (*L*) of the Natterjack toad *Epidalea calamita* in Belarus and Eastern Europe in general

Region	Source	Body length, *L* mm	
		♂♂	♀♀
USSR	Kuzmin, 1999	41,0-66,0	
Belarus	Pikulik et al., 1985	40,0-60,0	
Belarus	Novitsky, 2001	40,5-71,3 (56,9±0,38)	44,0-98,0 (63,9±1,56)
Belarus	Author's data	49,8-80,0 (62,1±0,5)	49,2-74,3 (62,4±1,22)

related coloration and pattern. In the analysis of population variability 15 parameters characterizing the linear sizes of the toads were used: *L* – body length; *L.cap* – length of head; *Lt.cap* – head width; *D.n.o* – distance from nostril to eye; *Sp.n* – distance between the nostrils; *L.o* – length of eye; *D.o.tym* – distance between the eye and the eardrum; *Lt.tym* – vertical diameter of the tympanic membrane; *L.tym* – horizontal diameter of the tympanic membrane; *F* – length of femur; *T* – tibia length; *D.p* – length of the first digit of the hind limb; *C.int* – length of the inner calcaneal tuber; *L.par* – length parotid; *Lt.par* – width parotid. Measurements were performed with calipers accurate to 0,1 mm.

On the base characteristics of the linear sizes 9 indexes, representing body proportions of the Natterjack toads, were calculated: *D.p*/*C.int*, *L*/*T*, *F*/*T*, *T*/*C.int*, *L.par*/*L*, *L*/*m*, *L.par*/*Lt.par*, *L*/*Lt.cap* and *L*/*F*+*T*.

Statistical parameters were calculated in the software packages Microsoft Excel and Statistica 6. At the sex dimorphism analysis in the morphological parameters the criterion of Student used. The reliability level was set to $p < 0,05$.

For the analyzing morphological variability of the Natterjack toad population in Belarus the sample including 165 adult males, 125 adult females and 350 immature specimens (n=640) have been collected. Minimum body size of the mature toads was determined on the individuals which participated in mating. To assessment sex structure of the population and life duration of the Natterjack toads in natural conditions individual tagging of amphibians was conducted using amputation of the terminal phalanges of fingers front and rear legs. The sex ratio in the population was assessed during the active period, from mid-April to early September.

RESULTS AND DISCUSSION

Statistical analysis of the data collected had shown that most of the external morphology parameters of the Natterjack toad in Belarus, according to the value of the coefficient of variation, characterized by a low variability (Table 1). Only a few characteristics, e.g., *L.o.tym*, *Lt.tym*, *D.p*, *Lt.par*, ranging within a narrow band (CV>20).

The comparison of the finding and literature data showed that most of studied morphometric traits of the Natterjack toads in this area of the range varies over a wider range than indicate other authors for this region (Bannikov et al. 1977, Pikulik 1985, Bakcharev 1986, Gruodis et al. 1986, Bakharev et al. 1995, Novitsky 2001) (Table 2). These differences are explained, most likely, a large size of the collected sample.

The maximum body length of *E. calamita* in Belarus reaches 80,0 mm. The size of adult males ranging from 49,8 to 80,0 mm (average of 62,1±0,5), and of adult females varies from 49,2 to 74,3 mm (62,4±1,22) (Table 1). The largest male was caught in spawning period in late April 2009 in the Stolbtsy district, the Minsk region (West Belarus) in one of the monitoring pond this species.

Data analysis showed that the Natterjack toad in Belarus has the biggest body size in the entire region of Eastern Europe. Sergius Kuzmin in his generalizing review of the amphibians the Soviet Union noted that the maximum size of this species across Russia, Ukraine, Belarus, Lithuania, Latvia and Estonia is limited 66 mm. Specified by Ruslan Novitsky very big size of the females (until 98 mm) in Belarus (Novitsky, 2001) was a mistake (personal message by R. Novitsky). Big size of the Natterjack toads recorded by the author of this article in study area is explained not only a large sample size, but some of the originality regional population.

In relation to very long breeding season the metamorphosis time of this amphibian in the study area varies greatly which affects the growth rates of toads and size-age structure of its population. For example, in Central Belarus juveniles appear from mid-June to early August. Body length of metamorphic specimens in

the first days of life on land, according to the observations of 18-22 June 2007, ranged from 7,5 to 17,3 mm (14,1±0,37) (*n*=45). Two weeks later, on July 3-5, their length was 13,5-25,3 mm (18,2±0,23) (*n*=28) and at the end of the season in early September, has reached 23,5-37,0 mm (28,1±3,6) (*n*=12). After 2 years, some individuals who appeared in earliest time reached the size of adult toads.

In accordance with body length (*L*) the population of the Natterjack toad in Belarus is divided into 3 distinct size-age groups (Fig. 2): 1) juveniles, *L* =7,5-37 mm; 2) immature under the age of 2 years (38-49,2 mm); 3) adults older than 2 years (over 50 mm). Long-term observation of the toads using individual markers showed that the lifespan of *E. calamita* in local environment usually does not exceed 6 years, during which until 4 reproductive cycles are observed. Toads, whose age was estimated at 7-9 years (according to the results of tagging), were recorded very few. The main group of the population, judging by composition of the collected sample, make up medium-sized specimens, body length which ranging in limits 56-60,9 mm.

Body weight is one of the most volatile morphological characteristics of the Natterjack toad in wild population of Belarus (CV is 25,8-29,7%), which is subject to clear sexual and seasonal variability. In adult specimens this parameter changes very widely, from 12,1 to 38,1 mm. On this basis distinct sexual dimorphism evident. Males are characterized by smaller values of mass than females. Most significant differences observed among males and females of the same size-age groups.

Sex dimorphism in the population is also found for the characteristics *L.o*, *Lt.tym*, *F*, *T* and *Lt.par*.

On indexes, representing the proportions of the body *E. calamita*, sex dimorphism appears only in some indices (Table 3).

In comparison with other species of toads the Natterjack toad has relatively longer limbs adapted jumping and short run. For instance, ratio of body length to length of tibia *L*/*T* of this species is for the males 2,89±0,02 and 3,1±0,04 for the females, while for the common toad *Bufo bufo* is 2.69±of 0.03 and 2.93±0,04, respectively, and for the green toad *Pseudepidalea viridis* is 2,64±0,02 and 2,79±0,04 (Drobenkov et al. 2005).

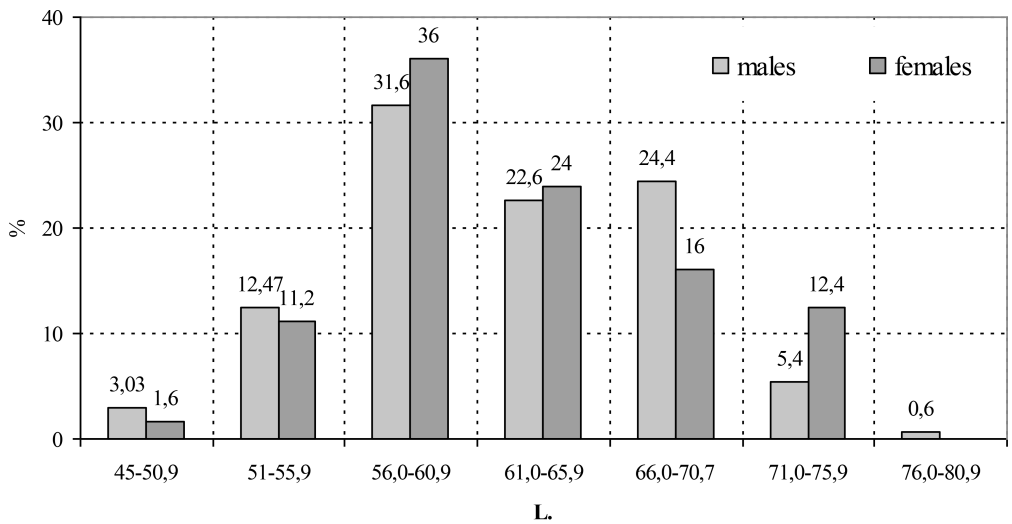

Fig. 3. The color differences of males and females Natterjack toad Epidalea calamita in population of Belarus.

Table 3. Variation of body proportions the Natterjack toad *Epidalea calamita* in Belarus

Index	sex	min-max	X±*m*	CV	t, *P*
D.p/C.int	♂♂ ♀♀	0,46-1,93 0,8-1,3	1,04±0,03 1,0±0,05	27,6 19,2	1,82, p<0,05
L/T	♂♂ ♀♀	2,06-3,23 2,7-3,2	2,89±0,02 3,1±0,04	5,7 5,5	1,96, p<0,05
F/T	♂♂ ♀♀	0,66-1,2 0,9-1,4	1,04±0,01 1,1±0,03	6,5 10,5	1,87, p<0,05
T/C.int	♂♂ ♀♀	4,7-12,2 5,0-7,9	7,3±0,2 6,4±0,03	21,3 15,1	3,43, p>0,99
L.par/L	♂♂ ♀♀	0,1-0,2 0,1-0,2	0,16±0,002 0,16±0,01	13,9 14,8	1,11, p<0,05
L/m	♂♂ ♀♀	2,17-4,35 1,9-3,5	2,83±0,06 2,5±0,16	16,6 21,3	3,12, p>0,99
L.par/Lt.par	♂♂ ♀♀	1,2-2,76 1,2-2,5	1,71±0,03 1,8±0,1	16,9 21,4	2,35, p>0,05
L/Lt.cap	♂♂ ♀♀	2,51-3,5 3,0-3,5	3,1±0,02 3,2±0,05	6,2 5,4	2,11, p>0,05
L/F+T	♂♂ ♀♀	1,24-1,61 1,3-1,6	1,41±0,01 1,5±0,02	5,5 6,2	2,48, p>0,05

Fig. 3. The color differences of males and females Natterjack toad *Epidalea calamita* in population of Belarus.

The coloration of head and upper body in the Natterjack toads in the study area had been painted usually light green, olive, or light gray. Males are often colored green, females in gray or olive (Fig. 3). On the upper body from the nose to the red-footed falcon thin specific dorsomedial stripe is located. Dorsal part of the body stained dark spots of irregular shape and small rounded orange specks. The edge of the upper jaw near the mouth franked with a light strip. Iris is emerald green.

Ventral part of the body is lighter colored than its dorsal surface; it is usually white or light gray color. Throughout the abdomen there is a small rounded gray spots. Abdomen color exhibits clear sexual dimorphism in this species. Throat and ventral surface of the body in the adult females are lighter, usually milky-white color, while males have a darker, grayish-white color.

The color of upper body in the Natterjack toads is very variable. The location, number and form dark spots on the back are an individual and allow you to identify almost every specimen. Most individuals in the population of Belarus (>85%) have a bright band of orange or red spots on the side from the back of the head to the hind limbs.

One of the most variable characteristics of the Natterjack toad pattern is the species-specific dorsomedial strip. In most specimens (98,3%), the strip is discontinuous, which can be considered the norm. In Belarus, this species has from 1 to 7 gaps on the strip, usually 2-3. Sometimes some other deviation in the form and location of the strip noted. For instance, 2,3% of the adult toads had a «loop» in the central part of the back, representing a large closed curve in the left part of the body. Such deviation is, apparently, an inherited sign, because it was found with high frequency just in toads, breeding in the same pond.

The sex ratios in population of *E. calamita* in active period vary considerably, due to the difference of reproductive cycles and rhythms activity of males and females. During spawning in breeding ponds sex balance varies from absolute dominance of males at the beginning and end of the season to the ratio of 5:3 (males: females) at the egg-laying peak. However, the real balance between the sexes in the population, according to the results of tagging the toads, performed in several groups in the summer season, has a more balanced composition, with a slight predominance of males (1,1:1) (Fig. 4).

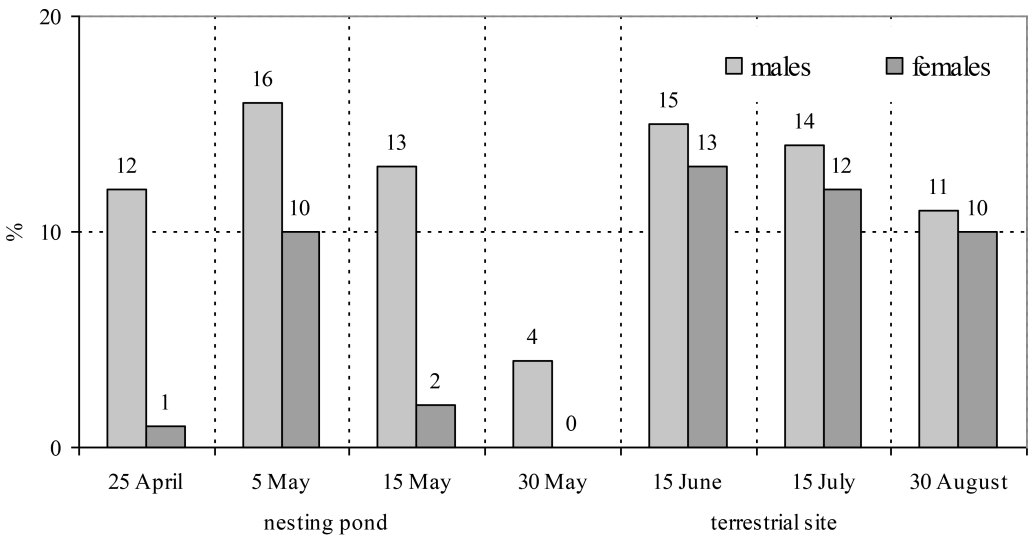

Fig. 4. Variation of the ratios males and females in the Natterjack toad *Epidalea calamita* population during the activity season in Belarus.

CONCLUSION

1. The obtained data characterize the Natterjack toad as the smallest toad species in the native amphibians, which differs by a stocky body and relatively long legs that contribute to short running.
2. Morphological features of the toad, such as green or grayish color, camouflage pattern, indicate to its adaptability to habitats both in terrestrial and aquatic environment.
3. For some of the analyzed characteristics a statistically reliable sex differences revealed.

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