DISTRIBUTION, SEX, SIZE AND MORPHOMETRIC STRUCTURE OF THE EUROPEAN COMMON SPADEFOOT TOAD (*PELOBATES FUSCUS* LAURENTI, 1768) IN THE AREA OF THE ZLATIYA PLATEAU

Yanitsa Ivanova[*], Teodora Teofilova[**], Nikolay Kodzhabashev[***]

[*] *Forestry University, Faculty of Forestry, Faculty of Ecology and Landscape Architecture, 1756 Sofia, Bulgaria, 10 Kliment Ohridski Blvd.; e-mail: imp.eagle@abv.bg*

[**] *Institute of Biodiversity and Ecosystem Research (IBER), Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria; e-mail: oberon_zoo@abv.bg*

[***] *Forestry University, Faculty of Forestry, Department of Hunting and Game Management, 1756 Sofia, Bulgaria, 10 Kliment Ohridski Blvd.; e-mail: ndkodjak@abv.bg*

Abstract

European common spadefoot toad (*Pelobates fuscus*) is a night active, very secretive species and the knowledge about it in Bulgaria is limited. The study in the area of the Zlatiya plateau was done with the aid of 100 pitfall traps, set to establish the common faunistic diversity.

Fixed and examined were 45 specimens. All important for the tailless amphibians morfometric indexes and values were measured and analysed. The animals were divided in 5 groups (juvenile, male subadult, female subadult, male mature adults and female mature adults). Statistical processing of the morphometric data showed that the sample as a whole can be considered homogeneous with weak variation of the signs.

The results were analysed and commented in a comparative aspect with similar studies in other parts of the geographic range of the species.

Morfometric data, in combination with the sex and size structure of the population, were indicative of its status and its trends, which was of great importance in determining the conservation significance and the favourable conservation status of the species. The results of this survey were useful for the preparation of a pack of conservation measures.

Key words: *morphometrics, morphometric indexes, Common spadefoot toad, Zlatiya plateau*

Introduction

Amphibians are elements of many aquatic and terrestrial ecosystems, but as shown by various studies, their numbers decrease, mainly as a result of the economic activities and the negative human attitude (Kuzmin, 1995). The importance of the amphibians in the functioning of the ecosystems is quite substantial. In many biocoenoses the amphibian biomass is comparable to the biomass of birds and mammals, and often even exceeds it (Garanin, 1977). Great is the importance of amphibians in the feeding of the animals of all systematic groups and as regulators of the numbers of the invertebrates (Garanin, 1975, 1976; Bulahov et al., 1979, etc.). They play an essential role, being one of the main series of the food chains.

The rate of growth of the amphibians is determined mostly by the climatic conditions during the seasons of their activity. For the terrestrial amphibian species (proved for the spadefoot toad) main factor is the humidity of the environment, and for the aquatic it is the temperature. The rates of growth differ between the sexes, generations appeared in different years, as well as among the individuals. Growth in all amphibians is limited; for each population there are maximal sizes for the males and females. In older individuals is noted some reduction of the length of the body – "desiccation" (Panchenko, 1984).

Morphometry adds a quantitative element in the descriptions, which allows more accurate comparisons and description of various complex shapes and numerical juxtaposing between the different forms. Morphometric studies have as their objective the description of the shape of the objects in the simplest possible way, by removing the accessory information and thus facilitating the comparison between different objects. Morphometric researches are often applied for analyzing the fossil data and quantification of different manifestations in the ontogenesis of organisms or their evolutionary relationships.

Material and Methods

Subject and area of study

The object of this study is the European common spadefoot toad (*Pelobates fuscus* Laurenti, 1768; Amphibia: Anura: Pelobatidae) (**Figure 1**). It is a terrestrial species; during the day the toad hides in burrows in the ground, and at night comes out to feed. The spadefoot toad enters the water only for breeding. Very distinctive mark is the vertical pupil of the eye (a proof for the nocturnal activity). Another characteristic is that the species lacks a tympanic membrane.

According to Ananeva et al. (1998) the maximum length of the body in common spadefoot toads is 71 mm in their entire range, but for Europe it reaches 90 mm. According to Buresh & Tsonkov (1942) the Bulgarian specimens are long on average 47 – 53 mm. Mihov (2002) and Paspalev & Peshev (1960) point the maximum body length of 80 mm. After metamorphosis toadlets have a total length of 10 – 33 mm with weight up to 6 g. Sexual maturity occurs in the third year, when the body length in males becomes around 41 mm, and 43 mm in females. Sex ratio is relatively equal. In the wild spadefoot toads live up to 4 years.

The conservation status of the common spadefoot toad is the following: Law on biological diversity (Annex III), Habitat Directive 92/43/EEC (Annex IV), Bern Convention (Annex II), IUCN Red List (LC). It is a threatened European species (Eggert, 2002). Populations are declining in major part of their distribution area (Nöllert, 1997; Kuzmin, 1999; Eggert, 2002).

The species is widespread in Central and Eastern Europe, to Central Asia in east. There are isolated populations in the valley of the river of Po in Italy and in European Turkey. In Bulgaria it occurs in the areas along the Danube River and in some parts of the Northern Black Sea coast (**Figure 2**). There are also detached localities in Western Bulgaria, in Sofia Valley. The species is found up to about 700 m and prefers open or overgrown with shrubs areas with light crumble soils. It breeds in swamps, marshes, puddles, channels (Biserkov, 2007; Naumov & Stanchev, 2010).

In this study we caught specimens from different sampling areas in the Zlatiya site: the Zlatiya plateau, the right bank of the river Ogosta between the villages of Hairedin and Sofronievo; the steppe-like habitat on the shore of the Dam Shishmanov Val, the banks of the Danube River before the town of Kozloduy.

Figure 1. *Pelobates fuscus* from the Zlatiya plateau (© N. Kodzhabashev)

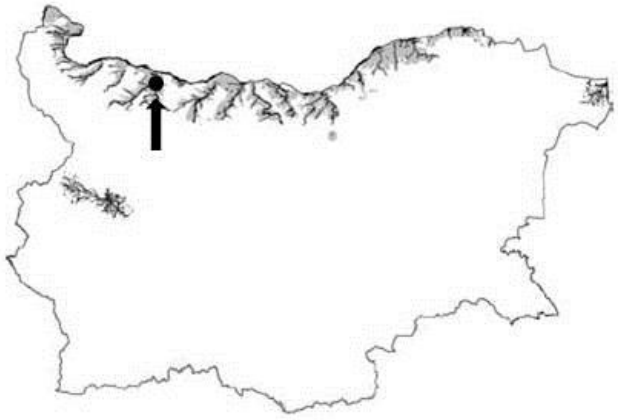

Figure 2. Distribution of *Pelobates fuscus* in Bulgaria (according to Biserkov, 2007). The arrow indicates the location of the research area (Zlatiya).

Methods of analysis

The toads are caught in pitfall traps with formaldehyde during a monitoring study of the biodiversity in the region.

We used the morphometric methods of analysis (**Table 1**). Morphometry of the animals is an area associated with studying the variation and changes in the shape and size of their body. Methods for extracting data include measurements of standard lengths and angles of the body or of different body parts (Terentjev & Chernov, 1949; Terentjev, 1950). The methodology is useful in cases where there are available linear and angular data, and is greatly helpful in studying the growth (Elewa, 2004).

Table 1. Measured morphometric parameters.

№	Parameter	Abbr.	Description
1.	Longitudo corporis	L.	body length
2.	Longitudo capitis	L. c.	head length
3.	Latitudo capitis	Lt. c.	largest head width
4.	Distantia rostri oculi	D. r. o.	snout lenght
5.	Spatium canthi rostralis	Sp. c. r.	distance between the front corners of the eye
6.	Distantia naris oculi	D. n. o.	distance between the nostril and the eye
7.	Longitudo oculi	L. o.	lengthwise diameter of the eye
8.	Latitudo palpebrae	Lt. p.	eyelid width
9.	Spatium palpebralis	Sp. p.	eyelid distance
10.	Spatium naris	Sp. n.	nostril distance
11.	Longitudo femoris	F.	thigh lenght
12.	Longitudo tibiae	T.	shin lenght
13.	Digitus primus	D. p.	length of the first digit on the hind limb
14.	Callus int.	C. int.	length of the metatarsal tubercle (“spade”)

The data were subjected to variation analysis in MS Excel. The most typical and common manifestations of the parameters are calculated as arithmetic mean value (average; Xav). We calculated the minimum (Min) and maximum (Max) dimensions of the individuals in the different age groups. In order to characterize the differences we used two indicators of dispersion (variance): standard deviation (SD), describing the extent of the variation of each variable values around the

mean value (Xav ± SD), and coefficient of variation (CV), which gives an opportunity to compare the variance of all parameters and to define the degree of homogeneity of the sample.

Tailless amphibians are growing throughout their whole lives. In order to achieve comparison of the results and to eliminate the influence of additional factors, such as the desiccation of the material, type and concentration of the fixation liquid, subjectivity of the measuring, etc., we have calculated 6 morphometric ratios.

Results and Discussion

We analysed a total of 45 specimens, of which 17 males, 20 females and 8 juvenile. The animals were separated in 5 sex-age groups: juvenile, male subadult, female subadult, male mature adults and female mature adults.

The results of the survey and analysis are presented in **Appendix 1**.

Statistical processing of the data shows that the sample as a whole can be considered homogeneous with weak variance of the features (CV < 12%). The exception is one of the sex-age groups – female adult spadefoot toads. This is resulting from the small number of measured individuals (10) on one hand, as well as the presence of females in reproductive phase, with spawn.

The coefficient of variation (CV, %) of the body length of the subadult toadlets is within the boundaries of the established for the young toadlets after completion of metamorphosis, when leaving the breeding ponds, in Saratov region (Yermokhin et al. 2015).

The comparison of the mean values of the main morphological parameters and the fluctuation around them with those of other parts of the spadefoot toad's areal, shows significant differences. The reason for this are the differences in the particular biotopes, metabolic processes, specific conditions of the predator-prey relations. Similar variations are found for Eastern Europe by Lada et al. (2005) and may be explained by the effect of stabilizing selection which maintains the optimum phenotype as a result of the adaptation to burrowing mode of life of this cryptic species (Borkin et al., 2003; Khalturin et al., 2003; Lada et al., 2005).

On the Balkans the southern border of distribution of the common spadefoot toad passes through Bulgaria. There are observations of increment of the values of the morphometric parameters north of it. For example, in southern Romania (Dobrudzha) the length of the body (L., cm) is averaging 4.2 ± 0.2 for the males and 4.8 ± 0.3 for the female individuals (Székely, 2010). These values are similar to the morphometry of the species in Zlatiya (4.7 ± 0.2 for the males and 4.9 ± 0.7 for the females). In northern Romania (Transylvania), however, the observed difference is evident: an average body length of 5.6 ± 0.3 for the males and 6.2 ± 0.2 for the females (Székely & Nemes, 2002).

The growth of the studied species is dependent on the humidity of the local microclimate, and in Dobrudzha the environment is dryer in comparison with the northern Romania. A similar pattern has been established also for measurements of the Syrian (Eastern) spadefoot (*Pelobates syriacus* Boettger, 1889) – Romanian specimens are on average bigger than the Bulgarian ones (Ugurtaş et al., 2002). These results may also be deriving from the presence of competition between these two pelobatid species, disturbance (anthropogenic), or variability of the trophic resources.

Sex ratio, represented by the participation of females (P_f) and the standard error (SE_{Pf}), where "n" is the total number of individuals, is the following:

$$P_f = n_f / n = 0.54 \qquad SE_{Pf} = \sqrt{P_f (1 - P_f) / n} = 0.08$$

In the present case, this ratio may be considered to be equal 1:1. This confirms the preliminary observations in the field, where we noticed that many of the studied individuals fall in pairs in the traps. It is also comparable with the ratio of the males: females (1:1.38) from Belarus (Drobenkov et al., 2005) and from the Carpathian region of Ukraine (ratio 1:1.44) (Pisanets, 2007).

Morphometry allows the analysing of the degree of sexual dimorphism in the species. Females generally have larger linear and angular dimensions, compared to males. This is especially clear in the comparison of the maximum values.

The arithmetic mean parameters for the adult specimens should not be considered as a criterion in this sample, as among the group of the females we established some specimens early entered upon a reproductive stage, which is an indicator of unfavourable environmental conditions.

The index of sexual dimorphism in length SDI (Size Dimorphism Index, proposed by Lovich & Gibbons, 1992), representing the ratio between the average length of the larger sex to the average length of the smaller sex, reduced with ± 1, tends towards equalized average values with a slight prevalence of the average length of the female adult specimens:

SDI (adult) = 0.03 SDI (subadult) = 0.99

Sexual dimorphism based on the size of the body is characteristic for the common spadefoot toad. It is in directly proportional relationship with the weight of the toads. In conditions of competition, where the reproductive success of the males is a function of the increased number of meetings with females, and in conditions of restricted trophic resources, probably the smaller males have an advantage, due to their need of less amounts of food. Furthermore, the larger females would have provided more spawn. At the same time, the SDI alters between the first and the second hibernation, and probably decreases with the increasing of the age of the anuran amphibians (Székely & Nemes, 2002).

The age structure of the studied micropopulation shows the preponderance of the sexually mature individuals (51.1%), followed by the subadults (31.1%), and the juvenile forms (17.8%). The ratio of sexually mature to a sexually immature is 1:1 for the females and 3:1 for the males.

Studied sample gives only limited information about the overall variation of the population of the common spadefoot toad, given its extensive range. Some of the existing potential threats to the species are the use of chemicals and preparations in the agriculture, the destroying of its habitats as a result of the annual ploughing, the drainage of the breeding places.

The studied species is one of the few amphibians found to successfully adapt to cultivable areas. The data about it in Bulgaria, however, are limited.

The plateau Zlatiya is defined as a Protected zone according to the Directive 92/43. This imposes the necessity of carrying out an extensive research of the populations of the common spadefoot toad, in order to prevent the decrease of their numbers on a European scale, as well as to provide for priority measures for its conservation in the Management plan of the Protected zone.

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Appendix 1. Statistical analysis of the morphometric parameters and ratios in the common spadefoot toad (*Pelobates fuscus*) from the plateau Zlatiya.

Sex/ Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	L.	L.c.	Lt.c.	D.r.o.	Sp.c.r.	D.n.o.	L.o.	Lt.p.	Sp.p.	Sp.n	F.	T.	D.p.	C.int.	L./ L.c.	Lt.p./ Sp.p.	L./ T.	F./ T.	D.p./ C.int.	Sp.c.r./ D.r.o.
juvenile																				
Xav., cm	3.13	1.07	1.19	0.43	0.61	0.29	0.43	0.23	0.41	0.27	1.30	1.14	0.47	0.26	3.00	0.61	2.79	1.16	1.78	1.38
SD_x±	0.22	0.09	0.12	0.05	0.05	0.03	0.03	0.03	0.04	0.02	0.11	0.07	0.04	0.02	0.22	0.09	0.10	0.06	0.12	0.16
Min, cm	2.83	0.97	1.02	0.36	0.53	0.24	0.38	0.18	0.37	0.24	1.16	1.01	0.40	0.23	2.77	0.42	2.49	1.07	1.66	1.32
Max, cm	3.51	1.22	1.43	0.50	0.66	0.32	0.50	0.26	0.47	0.28	1.49	1.22	0.53	0.29	3.18	0.70	2.92	1.28	2.00	1.78
CV, %	7%	8%	10%	11%	7%	9%	8%	11%	9%	7%	8%	6%	8%	9%	7%	14%	4%	5%	7%	12%
♀ **adult**																				
Xav., cm	4.88	1.50	1.83	0.67	0.88	0.40	0.60	0.36	0.53	0.42	2.13	1.79	0.74	0.42	3.27	0.68	2.75	1.19	1.78	1.33
SD_x±	0.70	0.21	0.24	0.11	0.12	0.04	0.05	0.06	0.07	0.06	0.40	0.32	0.14	0.08	0.18	0.13	0.17	0.09	0.07	0.15
Min, cm	3.85	1.15	1.30	0.53	0.67	0.34	0.50	0.21	0.41	0.30	1.37	1.30	0.51	0.29	2.99	0.41	2.46	1.05	1.67	1.10
Max, cm	6.06	1.83	2.25	0.88	1.07	0.44	0.65	0.41	0.64	0.50	2.72	2.30	0.94	0.54	3.51	0.90	3.02	1.39	1.93	1.57
CV, %	14%	14%	13%	17%	13%	9%	8%	17%	14%	15%	19%	18%	19%	20%	5%	19%	6%	7%	4%	11%
♂ **adult**																				
Xav., cm	4.74	1.47	1.81	0.66	0.90	0.40	0.58	0.35	0.55	0.43	2.03	1.76	0.74	0.42	3.10	0.62	2.74	1.17	1.77	1.37
SD_x±	0.18	0.09	0.10	0.06	0.05	0.04	0.03	0.03	0.05	0.04	0.05	0.06	0.07	0.03	0.23	0.09	0.13	0.06	0.10	0.16
Min, cm	4.46	1.29	1.64	0.58	0.76	0.35	0.53	0.31	0.45	0.38	1.95	1.63	0.62	0.34	3.05	0.56	2.46	1.11	1.63	1.18
Max, cm	5.05	1.59	1.97	0.79	0.94	0.49	0.62	0.40	0.63	0.52	2.10	1.81	0.82	0.45	3.48	0.73	2.90	1.20	1.90	1.62
CV, %	4%	6%	6%	10%	6%	10%	5%	8%	9%	9%	3%	3%	9%	8%	7%	14%	5%	5%	6%	12%
♀ **subadult**																				
Xav., cm	3.23	1.09	1.27	0.45	0.62	0.31	0.43	0.25	0.42	0.29	1.37	1.16	0.47	0.28	3.00	0.62	2.79	1.18	1.75	1.35
SD_x±	0.18	0.08	0.15	0.04	0.05	0.01	0.03	0.02	0.03	0.02	0.11	0.08	0.05	0.02	0.24	0.06	0.11	0.06	0.11	0.15
Min, cm	2.95	0.98	1.00	0.41	0.54	0.29	0.37	0.21	0.37	0.26	1.20	1.03	0.40	0.24	2.70	0.55	2.66	1.08	1.55	1.10
Max, cm	3.51	1.20	1.56	0.51	0.69	0.33	0.50	0.28	0.47	0.31	1.63	1.32	0.55	0.31	3.41	0.70	3.00	1.24	1.83	1.68
CV, %	6%	7%	12%	9%	9%	5%	8%	10%	7%	8%	8%	7%	10%	9%	8%	9%	4%	5%	6%	11%
♂ **subadult**																				
Xav., cm	3.25	1.11	1.30	0.45	0.59	0.30	0.46	0.26	0.41	0.29	1.41	1.21	0.48	0.28	3.00	0.62	2.77	1.16	1.74	1.35
SD_x±	0.22	0.12	0.17	0.02	0.07	0.02	0.04	0.03	0.03	0.03	0.08	0.02	0.04	0.01	0.26	0.05	0.14	0.06	0.11	0.17
Min, cm	2.93	0.95	1.04	0.43	0.49	0.27	0.42	0.23	0.37	0.26	1.31	1.19	0.44	0.26	2.74	0.62	2.44	1.09	1.57	1.11
Max, cm	3.44	1.22	1.39	0.46	0.65	0.33	0.52	0.29	0.45	0.33	1.49	1.24	0.52	0.29	3.19	0.66	2.78	1.24	1.82	1.51
CV, %	7%	11%	13%	3%	12%	8%	10%	10%	8%	10%	5%	2%	9%	5%	9%	8%	5%	5%	6%	13%