

VARIABILITY OF CERTAIN MORPHOLOGICAL CHARACTERISTICS IN *RANA ESCULENTA* (L.)

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Abstract: The paper presents results of investigations on the variability of certain morphological features in specimens of *Rana esculenta* (L.) in the population from Balții pond (Dorohoi, Botoșani County). Biometrical study consisted in determination of 12 morphological parameters and statistical interpretation of the results. It was stated a reduced variability of most of investigated parameters, as well as the possibility to discriminate the sexes function of arithmetical averages recorded for certain biometrical parameters.

INTRODUCTION

Rana esculenta (Linnaeus, 1758) belongs to the complex of green frogs, together with *Rana ridibunda* (Pallas, 1771) and *Rana lessonae* (Camerano, 1882). General opinion on the three species evidences the hybrid nature of *Rana esculenta* (L.) species, through expressing of some morphological characters which are intermediate to parental species of the complex. This is the reason for which the identification of the three species is widely used biometrical methods of such characters.

On other hand, global decline of the biodiversity, in this case of the Amphibians – about 200 species endangered and 32 species going to extinction (Blaustein and Wake, 1990; Alford and Richards, 1999; Houlihan et al, 2000) – assigns launching of monitoring programs of these species, actions implying the use of various investigation methods and techniques in order to get precise information on their situation, on the adaptive potential of Amphibians, etc. Genetic variability is an important feature in the evolution of a species, in its success in competition with other species, so we considered useful the approach of such a study on certain species of green frogs from ponds located in north of Moldova.

In this paper we intended to contribute to the knowledge of biodiversity of certain species of Amphibians, through the evaluation of variability of certain morphological characteristics in *Rana esculenta* (L.), using specimens caught from Balții pond located in Dorohoi city (Botoșani County).

MATERIAL AND METHODS

In order to achieve the proposed target, there were caught 53 individuals from *Rana esculenta* (L.) population (30 males and 23 females) from Balții pond located in a marsh area, with phreatic layers situated at low depths, in eastern part of Dorohoi city.

Sampling of specimens for our study was made with a special net. We observed that after the submersion of the net and attraction of the frogs with a colored floating piece, the efficiency of capture increased significantly. After biometrical measurements, all the specimens were released back into their natural environment. In order to prevent the eventual investigation of the same specimens if re-captured, they were marked before released in their environment. For marking, it was used the banderole method (Elmberg, 1989; Rice and Taylor, 1993 – cited by Cogălniceanu, 1997). For biometrical measurements it was used a digital sliding callipers (Brüder Mannesmann Werkzeugze GmbH, Germany) with 0.01 mm precision.

For setting up the samples, it was taken into consideration the necessity that all the data to be characteristic to adult population. In this sense, the considered criterion was the body length of caught specimens, as compared to mean values mentioned in Fauna R.P.R. – *Amphibia*, volume XIV, fascicle 1 (1960).

So, the sample of *Rana esculenta* (L.) included specimens with body length between 54 – (65) – 76 mm.

For biometric determinations there were considered the following morphological characters: interpalpebral distance (Sp.p.), eye length (L.o.), head breadth (Lt.c.), tympan length (L.tymp.), head length (L.c.), body length (L.), length of anterior limb (L.m.a.), length of thighbone (F.), length of shinbone (T.), length of tarsal articulation (L.tars.), length of metatarsal tubercle (C.int.), length of the first finger (D.p.).

On the basis of obtained data, it was determined the ratio between certain of the analyzed biometric parameters. From these, the most frequently used in literature (Wijnands and Van Gelder, 1976; Gubányi and Kórsós, 1992; Csata, 1998; Zamfirescu, 2002; Krizmanić, 2008) are:

- ratio between length of shinbone and the length of internal metatarsal tubercle (T/C.int.);
- ratio between the length of the first finger and the length of internal metatarsal tubercle (D.p./C.int.);

- ratio between the body length and the length of the first finger (L/D.p.).

For each studied morphological character was determined minimal and maximal values, arithmetical mean, standard error and variation coefficient. Interpretation of obtained results was made using comparative statistical tests. For determination of dispersion it was used Fisher-Snedecor test and, function of it, Student homoscedastic or heteroscedastic tests.

The results are presented in Tables 1 – 2 and in Figure 1.

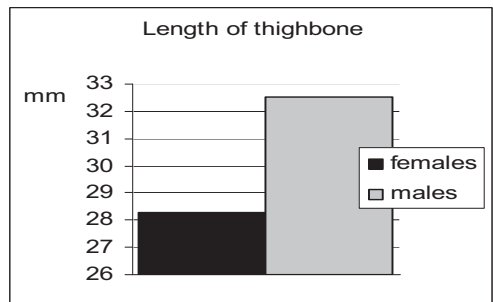
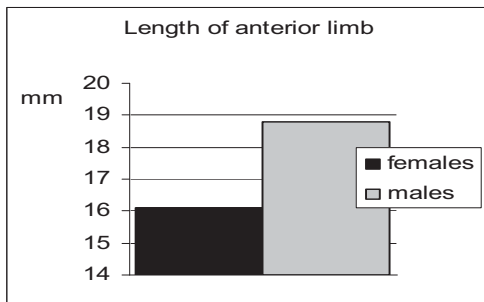
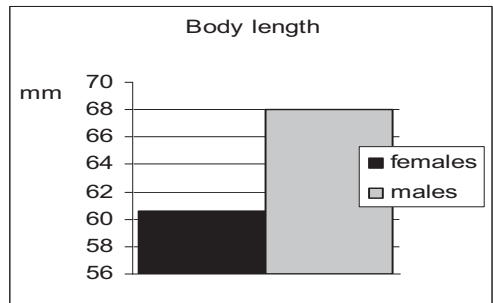
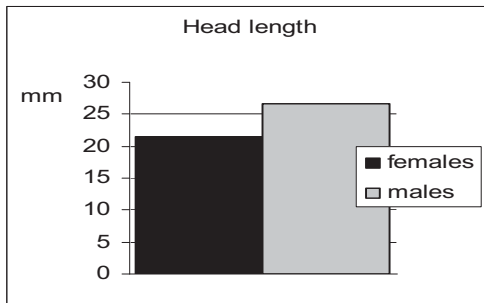
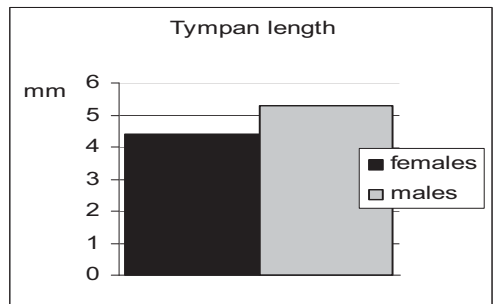
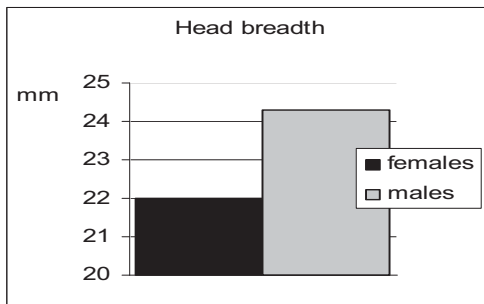
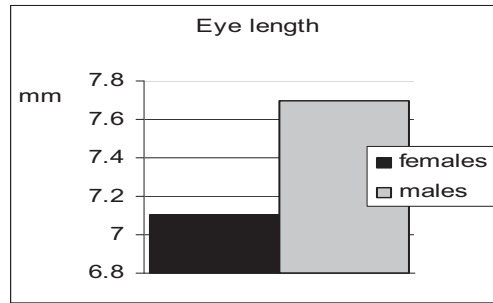
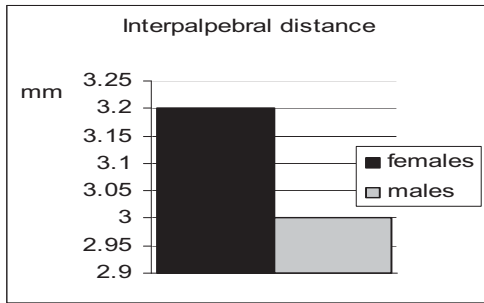
RESULTS AND DISCUSSIONS

After accomplishing biometrical measurements, the data were recorded for each parameter and, separately, for the two sexes, determining the values of statistical parameters for each of the morphological features (Table 1). In Figure 1 there are presented graphically the arithmetic averages of the investigated morphologic parameters in the two sexes.

Table 1 – Values of certain morphologic parameters (in mm) in *Rana esculenta* (L.)

Biometric parameter	Statistical parameter	<i>Rana esculenta</i>	<i>Rana esculenta</i> ♀	<i>Rana esculenta</i> ♂
Interpalpebral distance (Sp.p.)	Minimum	2,3	3,1	2,3
	Maximum	3,5	3,4	3,5
	Average	3,116	3,275	3,085
	Standard error	0,059	0,062	0,068
	Variation coefficient	9,49	3,81	10,08
Eye length (L.o.)	Minimum	6,4	6,4	6,4
	Maximum	9,0	8,1	9,0
	Average	7,676	7,150	7,776
	Standard error	0,125	0,417	0,120
	Variation coefficient	8,18	11,66	7,07
Head breadth (Lt.c.)	Minimum	19,8	19,8	22,2
	Maximum	26,7	24,7	26,7
	Average	23,964	22,000	24,338
	Standard error	0,339	1,237	0,278
	Variation coefficient	7,08	11,25	5,23
Length of tympan (L.tymp.)	Minimum	4,0	4,0	4,4
	Maximum	6,4	5,5	6,4
	Average	5,176	4,450	5,314
	Standard error	0,119	0,352	0,103
	Variation coefficient	11,51	15,82	8,93
Head length (L.c.)	Minimum	19,1	19,1	23,4
	Maximum	30,6	24,8	30,6
	Average	25,816	21,500	26,638
	Standard error	0,546	1,389	0,399
	Variation coefficient	10,57	12,92	6,86

Body length (L.)	Minimum	56,5	56,5	62,2
	Maximum	72,2	65,2	72,2
	Average	66,824	60,650	68,000
	Standard error	0,802	2,347	0,573
	Variation coefficient	6,00	7,74	3,86
Length of anterior limb (L.m.a.)	Minimum	15,0	15,0	15,7
	Maximum	21,4	18,3	21,4
	Average	18,376	16,150	18,8
	Standard error	0,323	0,779	0,275
	Variation coefficient	8,78	9,64	6,70
Length of thighbone (F.)	Minimum	26,9	26,9	28,6
	Maximum	38,7	30,7	38,7
	Average	31,904	28,300	32,590
	Standard error	0,586	0,889	0,567
	Variation coefficient	9,19	6,28	7,97
Length of shinbone (T.)	Minimum	24,8	25,9	24,8
	Maximum	33,6	28,6	33,6
	Average	28,988	26,800	29,404
	Standard error	0,421	0,625	0,433
	Variation coefficient	7,26	4,66	6,75
Length of tarsal articulation (L.tars.)	Minimum	14,3	14,3	14,3
	Maximum	18,4	16,6	18,4
	Average	16,120	14,950	16,342
	Standard error	0,264	0,551	0,274
	Variation coefficient	8,18	7,37	7,70
Length of metatarsal tubercle (C.int.)	Minimum	2,8	2,8	3,7
	Maximum	5,1	4,6	5,1
	Average	4,304	3,425	4,471
	Standard error	0,117	0,413	0,078
	Variation coefficient	13,63	24,11	7,98
Length of the first finger (D.p.)	Minimum	11,3	64,2	11,3
	Maximum	15,0	14,2	15,0
	Average	13,304	12,650	13,428
	Standard error	0,211	0,543	0,224
	Variation coefficient	7,92	8,59	7,65



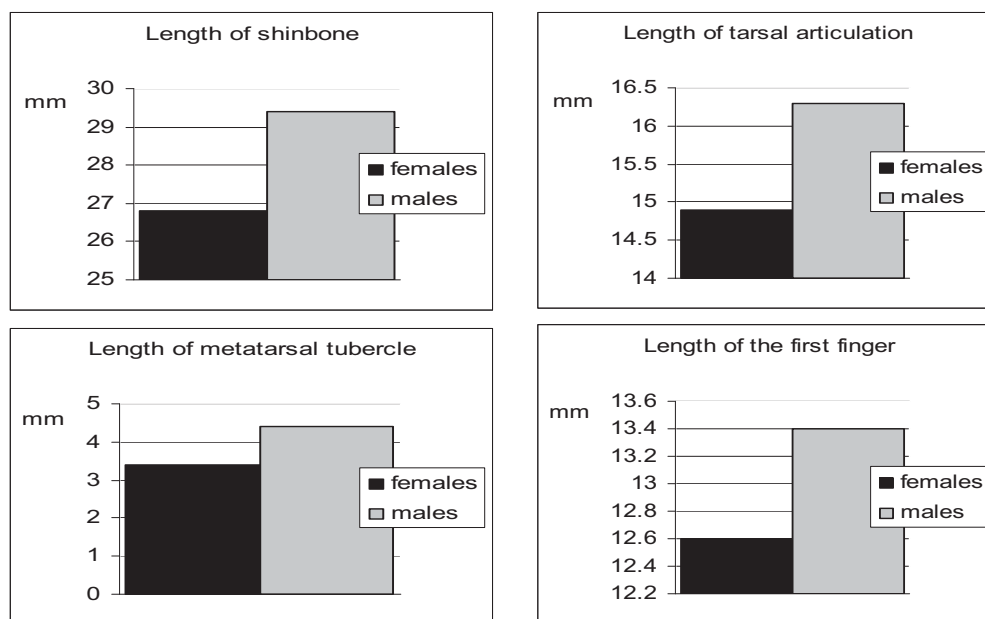


Figure 1 – Graphical presentation of average values of certain biometrical parameters in *Rana esculenta* (L.) function of the sex of specimens

Interpalpebral distance (Sp.p.) measured between eye balls, at the middle of the eyelids, ranged – in investigated *Rana esculenta* (L.) population – from 2,3 to 3,5 mm, with an average of $3,12 \pm 0,06$ mm, variability of the specimens in that population being small (VC = 9,49). Although the determined average of the interpalpebral distance is different for the two sexes ($3,27 \pm 0,06$ mm in females and $3,08 \pm 0,06$ in males), the possibility to use this biometrical parameter as criterion to differentiate the two sexes can not be considered, because T-test applied at significance level ($\alpha = 0,05$) confirmed the nule hypothesis (H_0) of the equality of the two averages.

Eye length (L.o), measured at the ends of the ocular longitudinal diameter, ranged in the sample of *Rana esculenta* (L.) population sample from 6,4 mm and 9 mm, with an average of $7,68 \pm 0,13$ mm, the variability of the parameter being low, both in males and females, according to the values of variation coefficient (VC under 10 %). T-test confirms the existence of a significant difference between the averages, at an error of 0,05; average of eye length is higher in male specimens as compared to females.

Head breadth (Lt.c), measured between mouth commissure, varies between 19,8 mm and 26,7 mm. Average value of this characteristic is $23,9 \pm 0,34$ mm, and variation coefficient (VC = 7,08) indicates a reduced variability of this parameter. Head breadth can not be used as discriminant parameter for sexes in *Rana esculenta* (L.) population, although males have superior averages as compared to females; statistical tests indicate that differences between the averages of samples are not significant.

Tympan length (L.tymp.) represented by the horizontal diameter of the tympan, ranged between 4,0 and 6,4 mm. Average tympan length, calculated for the whole sample, was of $5,18 \pm 0,12$ mm, and the variability of this characteristic is one mean (VC = 11,51). The average values

of tympan length differs significantly function of the sex of specimens; it is higher in males (significant T-test with significance level of 0,05).

Head length (L.c.) measured from the spike of mouth to mouth commissure is a biometric parameter whose values recorded in *Rana esculenta* (L.) population ranged between 19,1 and 30,6 mm, the average of this parameter in population was of $25,82 \pm 0,55$ mm, and the variability of the character was rather one mean to reduced (VC = 10,67). If considering the sex of specimens, no notable differences appears as concerns the dispersion of biometrical data (F test is not statistically significant); in turn, comparative analysis of arithmetic averages shows significant differences, the mean value of head length is bigger in males, with an error of 0,05.

Body length (L.) , measured from the spike of mouth to the anus, in *Rana esculenta* (L.) population, extended between following limits of variation : 56,5 - 72,2 mm, average length of the body being $66,82 \pm 0,80$ mm. Variability of this character was low (VC = 6,00). In the case of female specimens body length ranged between 56,5 and 65,2 mm, with an average of $60,65 \pm 2,35$ mm. In male specimens this character ranged between 62,2 and 72,2 mm, with a mean of $68,00 \pm 0,57$ mm. Comparative analysis of the samples, differentiated on sexes indicates a different dispersion of values in the two series of data (F test – significant – H_1), and the comparative analysis of the average values using heteroscedastic T-test shows a lower probability than significance level (0,05); this leads to the alternative hypothesis (H_1).

Length of anterior limb (L.ma.), measured from the articulation to the spike of the longest finger (finger III) within *Rana esculenta* (L.) population varied between 15 and 21,4 mm, with a mean value of $18,38 \pm 0,32$ mm, and the variability of the character was low (VC = 8,78). Arithmetical means differ function of sex, and statistical tests show significant differences (T-test), that confirms – at an error of 0,05 – a bigger mean in case of male specimens.

Length of thighbone (F.) measured from anus to the middle of the knee, is a parameter frequently used in the papers dealing with biometry of *Ranidae*. In investigated *Rana esculenta* (L.) population the values of this parameter varied between 26,9 and 38,7 mm, with an average for the whole sample of $31,90 \pm 0,59$ mm. The variability of this character is reduced (VC = 9,19), including the samples differentiated on sex. More, statistical analyses show a bigger value of thighbone length in male specimens with an error of 0,05.

Length of shinbone (T.) measured from the middle of knee articulation to the middle of the shinbone-tarsal articulation, varies between 24,8 and 33,6 mm, with a mean value, determined for sample of $28,99 \pm 0,42$ mm. Variability of this character is also low (VC = 7,26). Statistically, the difference between the averages determined for sexes is significant, the mean value being recorded in male specimens.

Length of tarsal articulation (L.tars.), measured from the middle of shinbone-tarsal articulation to the middle of tarsal-metatarsal articulation, ranged between 14,3 mm and 18,4 mm in investigated *Rana esculenta* (L.) population. Average length of the tarsus is $16,12 \pm 0,26$ mm, and the variability of this parameter is reduced (VC = 8,18). Statistical tests performed for the two sex-differentiated samples indicate equal distributions of the values, and average values of tarsal length are statistically different for the two sexes, with an error of 0,05.

Length of metatarsal tubercle (C.int.) represents a biometric parameter important for determination and species characterization in green frogs. Limiting values determined for this parameter were 2,8 – 5,1 mm, with a calculated average of $4,30 \pm 0,12$ mm. This character presented a mean variability (VC = 13,63). As concerns the possibility to differentiate the two sexes using as discriminant criterion the length of internal metatarsal tubercle, statistical tests showed significant differences between sexes, with an error of 0,05.

Length of first finger (digittus primus – D.p), measured from the spike to the distal end of internal metatarsal tubercle, represents an other morphological parameter very important in biometrical investigations of green frogs. In our studies, its values varied between 11,3 and 15 mm, arithmetic mean being $13,30 \pm 0,21$ mm. The variability of the character is low ($VC = 7,92$), being similar in female and male specimens. No significant differences were observed between the average values of the parameter for the two sexes.

A general assessment on the variability of the 12 morphological characters analyzed in *Rana esculenta* (L.) population showed that most of them presents a low variability, variation coefficient being generally under the level of 10. Only two of investigated parameters presented a mean variability: tympan length and the length of internal metatarsal tubercle. An other observation pointed that female specimens recorded, generally, a slightly increased variability of investigated parameters, with the exception of interpalpebral distance, length of thighbone, length of shinbone and of the tarsus, whose values were slightly increased in male specimens.

As concerns the ratio between certain of analyzed parameters (Table 2), the calculated values are similar to that mentioned in literature (Wijnands and Van Gelder, 1976; Gubányi and Korsós, 1992; Zamfirescu, 2002; Krizmanić, 2008). The most extended variability interval was observed in the case of T/C.int. ratio. Minimum value of this ratio was 5,69, maximum value was 9,25, and average value was $6,83 \pm 0,16$. Situation is the same in the case of analysis of this ratio for sexes. The other determined ratios varied in more reduced limits. So, the ratio D.p./C.int. varies between 2,63 and 4,21, with an average of $3,13 \pm 0,07$ for the whole caught sample; ($3,08 - 4,21$, with an average of $3,78 \pm 0,25$ in female specimens and $2,63 - 3,43$, with an average of $3,01 \pm 0,04$ in male specimens). L./D.p. ratio presented values between 4,59 and 5,69, with an average of $5,03 \pm 0,05$ in investigated *Rana esculenta* (L.) population; in female specimens limiting values were 4,59 and 5,09, with an average of $4,79 \pm 0,10$ when in male specimens limiting values were 4,66 and 5,69, with an average of $5,08 \pm 0,06$ mm. Comparative study of these ratios function of specimen sex shows not significant differences between mean values, with an error of 0,05. So, the values of these ratios don't allow sex differentiation, but may characterize the whole population of this species.

Table 2 – Values of the ratio between certain of analyzed biometric parameters

Number of specimens (<i>Rana esculenta</i>)	Statistical parameter	Ratio		
		T/C.int.	D.p./C.int.	L./D.p.
Total specimens (53)	Minimum	5,693	2,630	4,591
	Maximum	9,250	4,214	5,699
	Average	6,833	3,134	5,036
	Standard error	0,169	0,078	0,056
	Variation coefficient	12,36	12,44	5,63
Female specimens (23)	Minimum	6,217	3,086	4,591
	Maximum	9,250	4,214	5,095
	Average	8,071	3,786	4,799
	Standard error	0,687	0,258	0,106
	Variation coefficient	17,03	13,65	4,43

Male specimens (30)	Minimum	5,693	2,630	4,660
	Maximum	7,729	3,432	5,699
	Average	6,597	3,010	5,081
	Standard error	0,099	0,044	0,060
	Variation coefficient	6,91	6,81	5,45

CONCLUSIONS

Investigation of variability of 12 morphological characters in specimens of a *Rana esculenta* (L.) population from Balții pond (Botoșani County) showed that, excepting the interpalpebral distance, head breadth and the length of first finger, all the other parameters record average values different, function of sex;

Variability of investigated characters is reduced both at the level of analyzed sample, and function of sex. However, female specimens presented generally values of the variability coefficient slightly increased as compared to male specimens, in almost all investigated parameters;

Analysis of the ratio between certain of investigated parameters in *Rana esculenta* (L.) shows a low variability of the ratio between the body length and the length of the first finger (L./D.p.) in both sexes; so, this ratio is a positive indication for the characterization of the species *Rana esculenta* (L.).

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