



Original Contribution

LINEAR DIMENSIONS WITHIN POPULATIONS OF *PELOPHYLAX RIDIBUNDUS* (AMPHIBIA, ANURA, RANIDAE) FROM HABITATS WITH DIFFERENT LEVELS OF ANTHROPOGENIC TRANSFORMATION IN THE REGION OF PLOVDIV, SOUTHERN BULGARIA

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ABSTRACT

Major morphometric measurements (Length of body (L), Tibia (T), Femur (F)) in *Pelophylax ridibundus* from populations inhabiting two bodies of water in the outskirts of Plovdiv: one relatively clean and the other - polluted with pesticides, were analyzed. In both populations, independent of the characteristics of the living environment, there is clear sexual dimorphism - females are superior to males in size. When living in conditions of pesticide contamination, statistically reliable reduction of body size in both sexes of *P. ridibundus* is found.

Key words: linear morphometric parameters, sexual dimorphism, *Pelophylax ridibundus*, pesticide contamination, Southern Bulgaria.

INTRODUCTION

Within the variety of European green frogs *Pelophylax kl. esculentus* Linnaeus (1) in Bulgaria, the marsh frog *P. ridibundus* Pallas, 1771 is a widely spread species and inhabits the whole country; *P. essulentus* Linnaeus, 1758 is found in the north - along the Danube River, and *P. lessonae* Camerano, 1882 despite its supposed presence is not reliably documented (2). Morphometry of the lake frog in the country is not yet studied enough which creates the necessity of accumulation of factual material, especially considering the increased interest in this species regarding its use for bio-indication (3-5).

The aim of this study was the analysis of some basic morphometric measurements (Length of body (L), Tibia (T), Femur (F)) of *P. ridibundus*

from populations inhabiting ponds in the outskirts of Plovdiv regarding data filling on the dimensions of species inhabiting anthropogenically transformed environment, which has been presented in our previous works (6-7).

MATERIAL AND METHODS

The collection of the material was made in the spring of 2010 in two biotopes in Southern Bulgaria (**Figure 1**). The first - a water tank (regulated) at the beginning of a canal, which supplies a system of rice fields located 12-15 km. to North of Plovdiv. The reservoir is a water body of artificial origin, dimensions - 50-60 m. by 30-40 m. The water levels vary throughout the year and it is supplied by the lake "Orizare." Based on the data of physicochemical analyzes of water for the period 2000-2010 (8), this pond meets the second category (relatively clean) under the Water Act in the Republic of Bulgaria and Regulation № 7/08.07.1986, Ministry of Health. The first paddy fields are located not less than 4 km from the dam.

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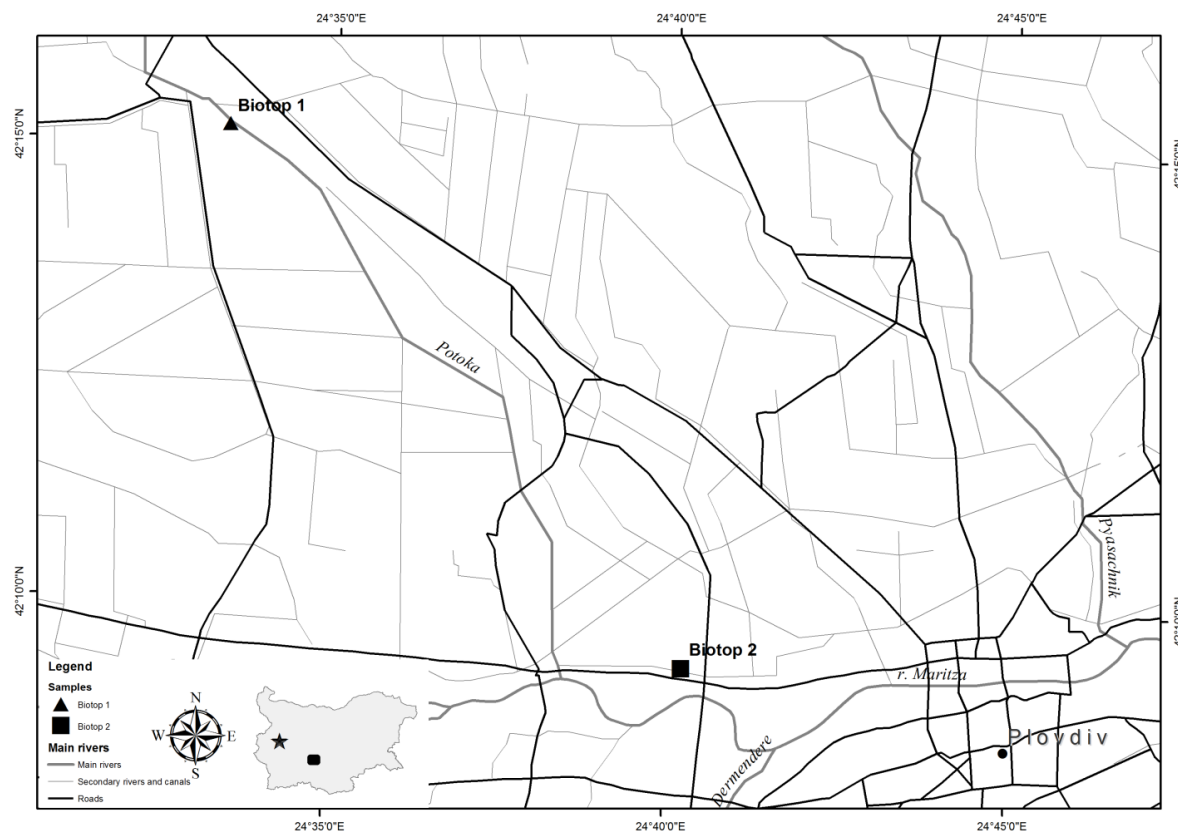


Figure 1. Geographical location of the studied water ecosystems

In our previous work (5), based on the bioindicational method a fluctuating asymmetry in populations of *P. ridibundus* the biotope has been assessed with the grade 1 according to which the environmental conditions are good for the development of the amphibians. In this work this is seen as basic. Here have been caught 75 (32 ♂, 43 ♀) individuals of *P. ridibundus* from population 1 (in the work are accepted contingent designation names).

The second habitat consists of several paddy fields located 5-7 km. to the West of Plovdiv. The rice paddies are ponds with a rectangular shape and dimensions of 250-300 m. The filling of the system is done in the spring through an irrigation canal with water from the river Chepelarska and it is emptied in September. After the rice paddies have been emptied in the autumn, animals from population 2 migrate and spend the winter in ponds remained from the old bed of the river Maritza located 3-4 km away from them. There are virtually year-long exchanges between rice fields and ponds for wintering purposes. Despite the variations in the

numbers of animals in them due to the nature of the water regime in rice paddies, population 2 is localized and isolated enough and its structural and demographic characteristics are not influenced by phenomena of migrational and immigrational nature (i.e. animals do not move away for long distances and outer influences are not unlikely).

On a regular basis, year-round in the rice paddies pesticides are imported. Here have been caught 78 (41 ♂, 37 ♀) individuals of *P. ridibundus* from population 2. Based on indicators fluctuating asymmetry (FA) the biotope receives the grade 4 - populations are in critical condition with impaired stability of development (5). In our work, habitat № 2 is regarded as anthropogenically contaminated.

The two researched biotopes are located from each other not less than 7-8 km. in a straight line (between them pass the highway "Trakia" and several minor roads), therefore we assume that exchange of animals between the two studied populations is impossible. In our opinion, the

significant distance of the ponds to the surrounding towns and villages and the lack of influence of anthropogenic factors, other than the direct deposit of pesticides in the rice fields, allows us to link the identified changes in population characteristics (in this case, morphometric) namely to the toxic effects of the pesticides.

The animals of both sexes are adults and sexually mature. The determination of the age was done while alive, based on body size (for all individuals $L > 60.0$ mm). Separation by sex was done directly based on the degree of development of secondary sexual characteristics (resonator bubbles in the corners of the mouth and blisters on the first finger in males).

Measurement of the standard morphological characteristics (L., F., T.) was conducted with callipers accurate to 0.1 mm using the commonly accepted methodology (9).

The statistical analysis was carried out by approved methods (10) with the help of the software package "STATISTIKA 6.0" and includes descriptive statistics (the normality of the distribution of the signs was tested with the Kolmogorov-Smirnov D-test and found a normal distribution: $p > 0.05$, which allowed a comparison of averages of investigated signs with a parametric Student's t -test).

RESULTS

The study of the basic linear parameters of *P. ridibundus* was performed in the following manner: first the values of the three metric dimensions as well as the indices L / F and L / T were analyzed separately for both sexes in each of the studied populations (**Table 1**), and then between individuals from the same sex the two populations (**Table 2**).

Table 1. Linear parameters of body and limbs of *Pelophylax. ridibundus* of the studied populations (range; mean \pm standard errors of means)

Population	Gender N	Length of the body L.(mm)	Length of the thigh F. (mm)	Length of the shank T. (mm)	L./F.	L./T.
1	♂ 32	(63.7–97.7) 76.86 \pm 2.80	(29.7–48.2) 36.33 \pm 1.74	(33.2–53.6) 39.39 \pm 2.01	(1.92–2.48) 2.13 \pm 0.04	(1.73–2.25) 1.97 \pm 0.04
	♀ 43	(91.3–100.7) 97.68 \pm 0.63	(38.9–46.8) 43.24 \pm 0.50	(40.2–49.31) 45.66 \pm 0.54	(2.15–2.38) 2.26 \pm 0.02	(1.97–2.27) 2.14 \pm 0.02
t		7.11*	4.47*	3.62*	4.25*	2.33*
p		<0.001	<0.001	<0.001	<0.001	<0.05
2	♂ 41	(62.5–72.3) 67.86 \pm 0.72	(27.5–32.9) 29.88 \pm 0.35	(30.2–35.3) 33.24 \pm 0.51	(2.06–2.48) 2.27 \pm 0.02	(1.60–2.25) 2.05 \pm 0.04
	♀ 37	(71.3–97.3) 83.29 \pm 2.05	(30.3–38.2) 34.17 \pm 0.90	(33.7–50.3) 38.23 \pm 1.28	(2.28–2.64) 2.44 \pm 0.03	(2.04–2.42) 2.19 \pm 0.04
t		7.23*	3.82*	3.01*	2.60*	3.40*
p		<0.001	<0.001	<0.01	<0.05	<0.001

Note: * statistically reliable difference for $\alpha = 0.05$

In both populations inhabiting the two biotopes in the region of Plovdiv, in all the three metric dimensions of the body and limbs, as well as both indices there was a clear gender difference: females outnumber males by size (**Table 1**).

However, comparisons between the populations showed that animals of both sexes in population 2 were statistically significantly superior in all three linear indices to those of population 1 (**Table 2**).

Table 2. Comparisons between the two populations in the main linear parameters.

Indices	Sex	Compared populations	t	p
L. (mm)	♂	1–2	3.11*	<0.01
	♀	1–2	6.72*	<0.001
F. (mm)	♂	1–2	3.64*	<0.001
	♀	1–2	8.81*	<0.001
T. (mm)	♂	1–2	2.97*	<0.01
	♀	1–2	5.35*	<0.001
L. / F.	♂	1–2	3.5*	<0.001
	♀	1–2	4.5*	<0.001
L. / T	♂	1–2	1.33	>0.05
	♀	1–2	1.25	>0.05

Note: * statistically reliable difference for $\alpha = 0.05$

DISCUSSION

On the Balkans (in Serbia), the morphological variability of green frogs from *Pelophylax cl. esculentus* is analyzed based on 10 morphometric attributes and 18 indices. A clear distinction between *P. ridibundus* and *P. lessonae* based on the indices T/Cint; DpPp/Cint; L/T; F/T; F/Cint and L/Cint was found out. At the same time *P. esculentus* occupies an intermediate position (11-12).

In the literature concerning this question, it has previously been noted that in *P. ridibundus* there are geographical differences in the expressions of sexual dimorphism in various morphometric features (13). The author has analyzed 11 samples and found that not only the degree of expression of gender differences, but even their orientation is not the same across different populations. This situation is illustrated by studies of other herpetologists. As for example, in the central part of Russia (Ivanovskaya area) sexual dimorphism in the body dimension in *P. ridibundus* was not found (14). According to Peskova (15), concerning more complex types of indicators, morphological-physiological sexual dimorphism in the Western Predkavkazie region in the Russian Federation is not found (most commonly, differences between the sexes are connected to the linear dimensions of the body). In our previous work in Bulgaria (3), was found out that living in conditions of pollution, females of *P. ridibundus* appear to be larger than males. In a later work (7), sexual dimorphism (female dominated) in complex morphometric features in the populations of *P. ridibundus* was found both in relatively clean and in polluted biotopes.

As noted above, we have a reason to consider the biotope inhabited by population 1 as relatively clean and the one inhabited by population 2 as anthropogenically contaminated. On the basis of the present data (Tables 1 and 2), it is apparent that in individuals of both sexes of *P. ridibundus* living in conditions of pollution (pesticide caused) there is a reduction present of the three main linear indicators (L., F., T.).

In the referent literature, there are data showing some reduction in size by the tailless amphibians inhabiting ponds with pesticide contamination. It has been associated with the accumulation of toxicants in the organism and with worsening of the food base in the water basins (16-18). The obtained data confirm the view of Peskova (15) that pesticide contamination unidirectionally causes a reduction in the body dimensions of amphibians inhabiting the water body.

CONCLUSIONS

1. Sexual dimorphism in populations of *P. ridibundus* in the region of Plovdiv was found on the main metric indicators of the body and limbs (L., F., T.) – females are fairly larger than males, both in the relatively cleaner and in the anthropogenically polluted biotopes.
2. The maximum body length in *P. ridibundus* populations in the region of Plovdiv (100.7 mm - females) is less than the maximum reported length for the country (120.2 mm – females near the town of Dimitrograd, southern Bulgaria (6).
3. In the anthropogenically polluted habitat (by pesticide contamination), a reliable reducing of the body and limbs size of *P. ridibundus* has been found.

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