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ORIGINAL ARTICLE

Features of the Biochemical Parameters of a Pendant Frog Blood in the Kazan City Lakes

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KEYWORDS

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ABSTRACT: This esearch was performed aimed to study the biochemical parameters of the pond frog (Pelophylax lessonae) blood, from the populations inhabiting three biotopes of the Kazan city. Individuals of the pond frog Pelophylax lessonae were caught in July 2018 in three biotopes: a secondary reservoir near the Victory Park in Kazan, the Maloye Glubokoe karst lake and the Krugloye lake, which is a conditionally control area (a reserve). Determination of blood biochemical parameters included the analysis of glucose, lactate, total protein and hemoglobin. Two indicators were studied among the hematological signs: the content of erythrocytes and leukocytes. A lower content of glucose, total protein and an increased level of lactate were found in pond frogs from the population of the city center (Victory Park) in comparison with the conditionally control area and the "green zone" of the city. There was low hemoglobin content among the frogs from urban areas. The individuals from the P. lessonae population living in the Victory Park reservoir (urban population) showed a state of hypoglycemia, an increased blood lactate content, total protein, and hemoglobin decrease, and the increase of specific number of erythrocytes, which may indicate the conditions of hypoxia caused by high level of pollution of urban habitats and eutrophication of water bodies. Probably, various adaptive reactions are observed in anthropogenically disturbed amphibian habitats, consisting in erythrocyte number increase in response to the hemoglobin level decrease and also the change in the metabolic process intensity.

INTRODUCTION

The response of living organisms to the transformation of the habitat is manifested in the morphophysiological state changes of an organism and individual organ systems [1, 2]. Biochemical parameters of peripheral blood, along with general hematological parameters, are most sensitive to stress effects of environmental factors and can serve as the indicators of the physiological status of animals and the stability of population development [3-5].

Among vertebrates, when choosing the objects for bioindication studies, amphibians have some advantages: high ecological plasticity, confinement to water bodies, relatively small migrations, and high abundance in wide range of habitat conditions [6-10]. Green frogs of the genus Pelophylax are most often used for this purpose due to their high abundance, high adaptive potential, fertility and population growth rate [11-15], which allowed them to spread in a variety of habitats, including urbanized areas.

In this regard, the study of green frog blood reaction to the effect of anthropogenic factors in water bodies of large cities is of particular interest. Therefore, this study was

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performed aimed to identify the biochemical parameters of the peripheral blood of the pond frog Pelophylax lessonae (Camerano, 1882) from the territory of the urban ecosystems of the Kazan city.

MATERIALS AND METHODS

The samples from the populations of the pond frog Pelophylax lessonae (Camerano, 1882) were served as the material for this study. The frogs were caught in three key areas (July 2018): 1. near Victory Park: a secondary reservoir formed as the result of groundwater damming (n = 18); 2. forest park "Lebyazhye": karst lake "Maloye Glubokoe" (n = 15); 3. on the territory of the buffer zone of the Volzhsko-Kamsky state natural biosphere reserve: Lake Krugloye (control site; n = 14). For the territory of Kazan, it is customary to distinguish four functional zones [3], of which two studied urban habitats belonged to the zone of multi-storey buildings (Victory Park, zone II) and the green zone of the city (Lake Maloye Glubokoe, zone IV).

Blood sampling was performed after decapitation. For hematological studies, tubes with EDTA were used, and for biochemical analysis they used the tubes with gel and an activator from Minmed Company.

The study of biochemical parameters included the analysis of glucose (Mm L^{-1}), lactate (mM $^{-1}$), total protein (mM L^{-1}) and hemoglobin (g L^{-1}) in the blood. Two hematological indicators were studied: the content of erythrocytes and leukocytes (the number of cells per liter of blood). A Nikon H550S microscope was usef for counting of blood cells.

Hydrochemical parameters were assessed using the standard methods [16, 17] on the basis of the Department of Environmental Engineering and Water Use of the Kazan (Volga Region) Federal University and the Volga-Kama State Natural Biosphere Reserve. The values of hydrochemical parameters (the content of ammonium ions, nitrates, phosphates, and ASA) were higher in the reservoirs of the Victory Park and Maloye Glubokoe Lake in comparison with the conditionally control area (Krugloye Lake) (Table 1).

Table 1. Hydrochemical parameters of the studied water bodies

Localities	pH (un)	Ammonium ion (mg l^{-1})	Nitrite (mg l^{-1})	Nitrates (mg l^{-1})	Phosphates (mg l^{-1})	ASSAS (mg l^{-1})
Victory Park Pond	7,6	2,0	0,00	0,75	0,12	0,095
Small Deep Lake	7,1	0,85	0,02	0,1	0,06	0,015
Round Lake	7,4	0,14	0,042	< 0,10	< 0,05	0,021

Nonparametric Kruskal-Wallis test was used to compare mean values . Multiple data comparisons were made using the rank test. Comparison of variances is done using Leuven's test. Pairwise comparison of variances was carried out using Fisher's test with Bonferroni's correction. Data were analyzed by Statistica 6.0 software (StatSoft Inc.).

RESULTS AND DISCUSSION

The obtained values of blood biochemical parameters and hematological parameters were shown in Table 2 and 3.

Table 2. Biochemical parameters of the peripheral blood among the populations of the pond frog Pelophylax lessonae in Kazan and the conditionally control habitat

Localities	Glucose (mM l^{-1})	Lactate (mM l^{-1})	Total protein (mM l^{-1})	Hemoglobin (g l^{-1})
Victory Park Pond	4,68 ± 0,34	7,14 ± 0,48	23,29 ± 0,68	72,63 ± 0,78
Small Deep Lake	7,71 ± 0,16	5,48 ± 0,17	34,15 ± 0,38	73,61 ± 0,78
Round Lake	7,67 ± 0,16	5,28 ± 0,16	37,99 ± 0,37	80,23 ± 0,61

Table 3. Hematological indices of peripheral blood among the populations of the pond frog Pelophylax lessonae in Kazan and the conditionally control habitat.

Localities	Erythrocytes, thousand billion of cells/l	Leukocytes, billions of cells/l
Victory Park Pond	2,63 ± 0,05	27,21 ± 0,72
Small Deep Lake	2,55 ± 0,04	26,96 ± 0,64
Round Lake	2,45 ± 0,04	28,88 ± 0,77

Kruskal-Wallis test was used to compare three populations of the pond frog in terms of blood biochemical parameters that revealed statistically significant differences in blood glucose ($H = 29.5$; $p < 0.001$), lactate ($H = 8.7$; $p = 0.013$), total protein ($H = 39.4$; $p < 0.001$), hemoglobin ($H = 28.0$; $p < 0.001$), the number of erythrocytes ($H = 7.3$; $p = 0.025$).

Further multiple comparisons using the rank test revealed detailed differences between populations. The glucose and protein content in the population from the Victory Park reservoir was lower than among the individuals from the other two populations (Figure 1, A and B). Also, the individuals of the population from the Victory Park reservoir were characterized by overestimated lactate values (Figure 1D). The hemoglobin content in the blood was higher among the population of Lake Krugloye as compared to the urban populations (Figure 1C).

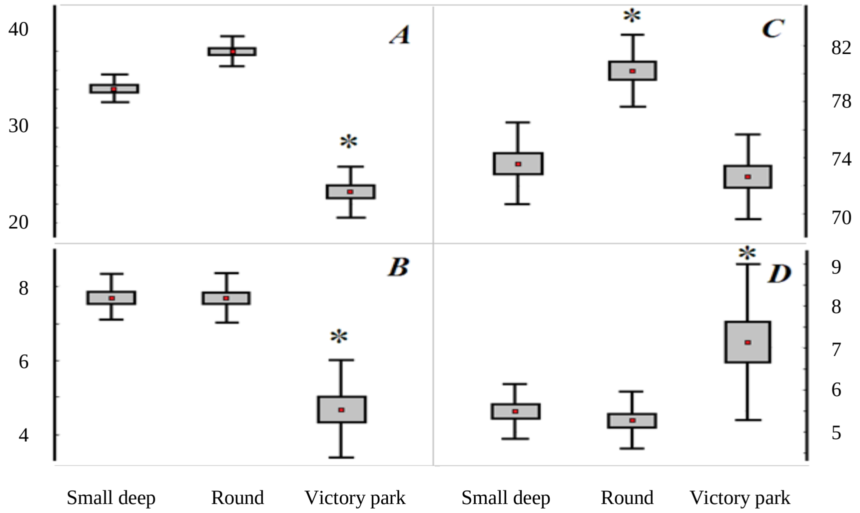

Figure 1. The content of total protein (A), glucose (B), hemoglobin (C) and lactate (D) in the peripheral blood of the pond frog Pelophylax lessonae from three populations inhabiting the water bodies of the Kazan city.

The mean, the mean error, and the standard deviation were given. The asterisk indicates statistically and significantly different groups ($p < 0.05$).

Multiple comparisons also revealed a significant decrease of erythrocyte number among the individuals from "Victory Park" as compared with the individuals from the control area (Round Lake).

Comparison of the variances of the studied blood biochemical parameters revealed statistically significant differences in the variances of glucose and lactate content. Pairwise comparison using the Fisher test showed variability increase among the individuals from Victory Park (zone II) as compared to other localities (Figure 1 A - D).

The state of hypoglycemia and total protein content decrease in the blood of individuals of the P. lessonae population from the "Victory Park" (belonging to the zone II of the Kazan city) indicates a possible imbalance of metabolism caused by various reasons (damage to internal organs due to parasitic invasion, body intoxication, etc.). The increased lactate content in the Victory Park population may indicate an increase of energy expenditure under the conditions of strong anthropogenic pressure. Similar conclusions were reported in previous studies on the basis of morphophysiological parameter assessment, oxygen consumption and the state of excitable tissues [18-20]. Probably, the high level of metabolic processes among the animals from the populations living under the conditions of intense anthropogenic pressure in an urban environment is an adaptive mechanism.

The decrease of hemoglobin level in the blood of P. lessonae and the increase in the specific number of erythrocytes may indicate hypoxic conditions caused by a high level of urban habitat pollution and eutrophication of water bodies. Apparently, an adaptive reaction was observed in the anthropogenically disturbed habitat of an urbanized area in the form of erythrocyte number increase against the background of hemoglobin decrease in the blood. In natural habitats, the efficiency of redox reactions is higher, and it is achieved without erythrocyte number increase, which was also noted by other studies [3].

CONCLUSIONS

The revealed tendency is presented as an adaptive response when living under the conditions of the natural environment anthropogenic transformation. The results obtained in this study are preliminary, and further research is needed to confirm the formulated conclusions in detail.

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Conflict of interest

The authors declared no conflicts of interest

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