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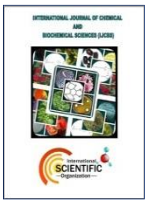

Functional features of acid-base balance in the moor frog (*Rana arvalis*) depending on environmental conditions in the Urals

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Abstract

Our study aimed to characterize the acid-base balance in order to evaluate hematological parameters and take them into account when evaluating the biophysiological state of the organism in order to prevent energy consumption. The acid-base balance of mature specimens of moored frog (*Rana. arvalis*) was studied. living in the Kalinovskie cuts, the South-Western Forest Park (Forest Park Zone) and Lake Kurtan, (Kurgan Region) in Yekaterinburg. Where the pH and Where the concentration of $ctCO_2$ was studied, pCO_2, concentration of bicarbonate ions $cHCO_3^-$, the concentration of blood buffer systems (excess of bases in whole blood— BE), (concentration of buffer bases— BB),(excess of deficit of standard blood bases— cSBE),hematocrit Hct,hemoglobin ctHb, Na^+, K^+ and Ca^{+2} in plasma**.** Significant differences in one of the blood parameters cNa, cK, cCa , Hct, $cHCO_3$, cBE, cBB and $ctCO_2$ in the compared samples of animals, in both habitats were revealed in the study. where the concentration of $ctCO_2$ increased in the blood of frogs living in the Kalinovsky cuts and Southwest, compared with samples collected from Kurtan. This increase increased $cHCO_3$-, cBE, cBB and Ca^{+2} levels. While the concentration of Na^+, K^+ and ctHb in the blood of frogs in Kurtan increased. In addition, statistically significant differences were observed in concentrations $cHCO_3$,cBE and $ctCO_2$ between morphs (Maculatus and Striata) for the Moored Frog.

Keywords: Acid-base balance of blood, Moored Frog, Electrolytes, blood gases, Blood Buffer Systems, Maculatus, Striata.

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1. Introduction

Studies have conclusively shown that amphibian colonies are subject to significant environmental stress from low pH environments. These results fluctuate depending on the stage of development and depend on complex interactions with various biological, chemical and physical factors [1]. Acid-base status is a highly regulated physiological process resulting from the balance of ions in the body, referred to as acid-base. The effectiveness of regulatory systems largely determines the compensatory changes in pH for a given disturbance. Vertebrates minimize or compensate for acid-base disturbances through general processes that include ion transport and pCO_2 changes. pH regulation in amphibians is difficult because there are many potential sites for gas and ion exchange with the environment [2]. Acid base is achieved by replacing the skin, lungs, and bladder with related ions while regulating acid-base balance and altering plasma bicarbonate levels. Some of the ion exchange mechanisms involved in the regulation of osmosis in these animals include the transfer of acid-base ions between the animal and the environment [3].

Acid-base regulation in animals occurs through three main mechanisms that work in concert and on different. timescales to regulate pH. The first, fast-acting regulation of intracellular pH is based on biochemical, cellular and molecular adjustments that are based on pre-existing concentrations and gradients of hydrogen (H^+), bicarbonate ions (HCO_3^-) and electrolytes contained in body fluids to regulate pH [4]. The second mechanism, regulation of airway acid-base balance, involves adjustment in the ventilation rate, increasing or decreasing arterial CO_2 partial pressure (PCO_2) with consequent effects on fluid pH [5]. The third and slowest mechanism is acid-base. regulation by excretory organs. This latter mechanism usually involves the excretion or retention of concentrations of bicarbonate, carbonic acid, and electrolytes and/or the excretion of waste products contained in body fluids [6]. To prevent changes in the pH of the medium when acids or bases are added to it, buffer systems are necessary. Most buffer systems in the blood are bicarbonate, phosphate, protein, and hemoglobin. The first three systems play a particularly important role in blood plasma, and the hemoglobin buffer, the most powerful one, acts in erythrocytes [7]. There are several buffer systems that reversibly bind hydrogen ions and prevent any change in pH. The bicarbonate buffer system (the strongest and most controllable among buffer systems) is of particular importance: excess protons (H^+, hydrogen ions) react with bicarbonate ions (HCO_3^-) to form carbonic acid (H_2CO_3). Subsequently, a decrease in the amount of carbonic acid occurs as a result of accelerated release of carbon dioxide (CO_2) as a result of hyperventilation of the lungs (the concentration is determined by the pressure in the alveolar gas mixture). Almost all chemical reactions in the body depend on maintaining the concentration of hydrogen ions within physiologically acceptable limits [8]. Biochemical, cellular and molecular reactions are the fastest. Blood, as the internal environment of the body, is known to be sensitive to harmful environmental factors; therefore, various hematological parameters can be used to assess the physiological state of animals. To date, the specificity of blood parameters for amphibians, especially representatives of the genus *Rana* living in mineral (salty) environments, has been studied in a relatively small number of works . *R. arvalis* is a ranid frog found in the western Palearctic in a wide range of habitats and acidity levels [9]. Breeding occurs in early spring, at higher latitudes shortly after the spring snowmelt, which coincides with the acidity peak in acidic areas. the moored frog is brown, usually with a clear greenish tint, dark spots and dots. there are also several typical patterns that are genetically determined,is a polymorphic species, in some individuals there is a clearly expressed light dorso-medial stripe (“striata” morph) reaching the end of the muzzle[9].

2. Materials and methods

A comparative study of the specifics of maintaining the acid-base balance of the blood of the moored frog in the Middle Urals in 2 populations living in habitats differing in conditions was undertaken. Samples of sexually mature moored frogs were selected in the vicinity of Yekaterinburg (Kalinovskie cuts, the South-Western Forest Park (Forest Park Zone), n=59) and (Lake Kurtan - Kurgan region, n=41). Special permission to catch representatives of this species in Russia is not required. After capture, the animals were taken to the laboratory for research. Before blood sampling, amphibians were euthanized with propofol in accordance with the regulations of the Russian Federation of 1977 and the second part of the report of the EU DGXT working group (1997). After euthanasia, Blood samples were taken directly from the heart through a ventricular incision using a small needle filled with heparin. The study of blood gases and electrolytes of whole blood of amphibians was carried out using a GASTAT-navi analyzer (Japan). A sample of whole blood with a volume of 200 μl was used for the study (the analysis time was 165 s). The following parameters were obtained by direct potentiometric measurement: pH - ion concentration (activity) H^+, PCO_2 - partial pressure of CO_2, Na^+ - sodium ion concentration, K^+ - potassium ion concentration, Ca^{2+} - calcium ion concentration, - hemoglobin concentration, Hct - hematocrit. Calculated parameters: HCO_3- - bicarbonate concentration, pCO_2 - total CO2, BE - excess (or deficiency) of bases, Hb - hemoglobin concentration, BB - sum of bases of all blood buffer systems, SBE - standard base excess. Hydrochemical analyzes were performed in the laboratory of physical and chemical analyzes of the Ural State Mining University, as well as in the laboratory of engineering and environmental testing of AquaSolum LLC. Statistical data analysis was performed using the Statistica for Windows software package.

3. Results and Discussions

Many studies convincingly demonstrate that low pH conditions have an important ecological impact on amphibian communities. These effects vary across development and depend on complex interactions with other physical, chemical, and biological parameters. Acid sensitivity varies widely within and among amphibian species. The acid-base balance of mature individuals of the moored frog (Rana. arvalis) was studied. living in the Kalinovskie cuts, the South-Western Forest Park (Forest Park Zone) and Lake Kurtan, (Kurgan Region) in Yekaterinburg. Lake Kurtan, (Kurgan Region) - a biochemical district (with a very high content of minerals) Comparative analysis of the state of the aquatic environment of habitats revealed a number of significant differences in 2014/2022 (Table 1). Thus, the total mineralization is more than higher (929.4±158.2 mg/l) in the lake. Kurtan, Kurgan region, where there is a significant increase in the concentration of potassium, sodium, chloride ions, as well as an alkaline pH of 7.97 (Table 1), while in the Forest Park zone a slightly acidic pH is noted, characteristic of the natural waters of this region . The differences are due to the fact that since the territory of the Kurgan region is a highly mineralized (saline territory), where a significant amount of mineral substances accumulate. It is to be expected that physiologically important elements such as Ca and Na will influence the pH tolerance of amphibians. Therefore, some of them may also be of interest for amphibian conservation work in acidified areas [10].

The acid-base and ionic balance of the blood depends on the relative activity of the epithelial Na^+ and Cl^- carriers. the Na^+/H^+ exchanger is the predominant epithelial transporter influencing the acid-base state, while the Cl^-/HCO_3^- exchange is less important. Cl^- and Na^+ transport are important components influencing the acid-base state. Serotonin (5-hydroxytryptamine, 5-HT) stimulates Na+ and Cl^- transport. the influence of exogenous serotonin is four times greater on Na^+/H^+ metabolism than on Cl^-/HCO_3^- transport, which leads to an increase in acid secretion and an increase in blood pH. The anionic component of blood consists of both Cl^- and HCO_3^-, and these ions are interchangeable over a wide range of concentrations [11].

Obviously, in animals living in conditions of increased mineralization (Lake Kurtan), there is a disruption in the functioning of the bicarbonate buffer system, which is expressed in functional changes in potassium-sodium transport and, against this background, In the population of the forest park zone, a low level of hemoglobin was noted (ctHb =10.1±0.4), in comparison with the population of Lake Kurtan (ctHb =11.1±0.5) (Table 2). It can be assumed that this is due to the functional load on the hemoglobin buffer system under conditions threats of alkalosis. In alkalosis in the lungs, hemoglobin behaves like an acid (oxyhemoglobin HHbO2 is a stronger acid than CO2), which prevents the blood from becoming alkaline.

Table 1: Hydrochemical indicators of the studied habitats (2016-2022)

The habitats	Cl^-	$S0_4^{2-}$	Na^+	K^+	**pH**	**Mineralization (mg/l)**
forest park zone	12.5±153.8	20.5±2.9	3.28±19.10	4.48±1.2	6.68±0.2	119.7±61.3
Kurtan - Kurgan region	703.9±165.5	15.1±6.7	204.7±46.3	11.4±2.6	8.3±0.3	1585.1±267.7
P	p=0.001	p=0.5	p=0.001	p=0.03	p=0.0003	p=0.0001

The habitats of the studied populations differ significantly from each other, since the territory of the Kurgan region is a highly mineralized (saline territory), where there is a significant increase in the concentration of potassium, sodium, chloride ions, as well as an alkaline pH of 7.97.

Table 2: Parameters of acid-base balance in two samples of *Rana arvalis*

pH	**pCO_2, torr**	**cNa^+, mmol/l**	**cK^+, mmol/l**	**cCa, mmol/l**	**Hct (%)**	**ctHb, g/l**	**$cHCO_3$, mmol/l**	**cBE, mmol/L**	**cBB, mmol/l**	**cSBE, mmol/l**	**$ctCO_2$, mmol/l**
forest park zone (n=59)											
7.46 ± 0.01	13.2 ±2.06	115.4±1.14	4.96 ± 0.17	0.67 ± 0.02	30.1± 1.62	10.0± 0.40	8.88±0.87	-13.1±0.98	32.9± 1.07	-13.4±1.35	9.25± 0.91
Kurtan - Kurgan region (n=41)											
7.41 ± 0.02	16.1± 2.47	122.0 ±1.47	5.76 ± 0.22	0.56 ± 0.03	35.4 ±1.94	11.0 ±0.49	4.66 ±1.07	-18.7±1.19	28.0± 1.29	-17.2±1.62	4.93± 1.12
Significance of differences											
p=0.06	*p*=0.36	*p*=0.0006	*p*=0.006	*p*=0.003	*p*=0.03	*p*=0.11	*p*=0.002	*p*=0.0004	*p*=0.003	*p*=0.07	*p*=0.003

Significant differences in one of the blood parameters cNa, cK, cCa , Hct, cHCO3, cBE, cBB and ctCO2 in the compared samples of animals, in both habitats were revealed in the study.

Table 3. Differences in blood gas and electrolyte concentrations between morphs

Parameter	**Maculatus(0)**	**Striata(1)**	**Significance of differences**
$cHCO_3$	8.5±0.9	5.6±1.04	p=0.04
cBE	5.6±1.04	-8.5±9.9	p=0.04
$ctCO_2$	8.9±0.97	5.9±1.1	p=0.04
N	59	41	

The striata has less bicarbonate ion, base excess, and carbon dioxide than maculatus.

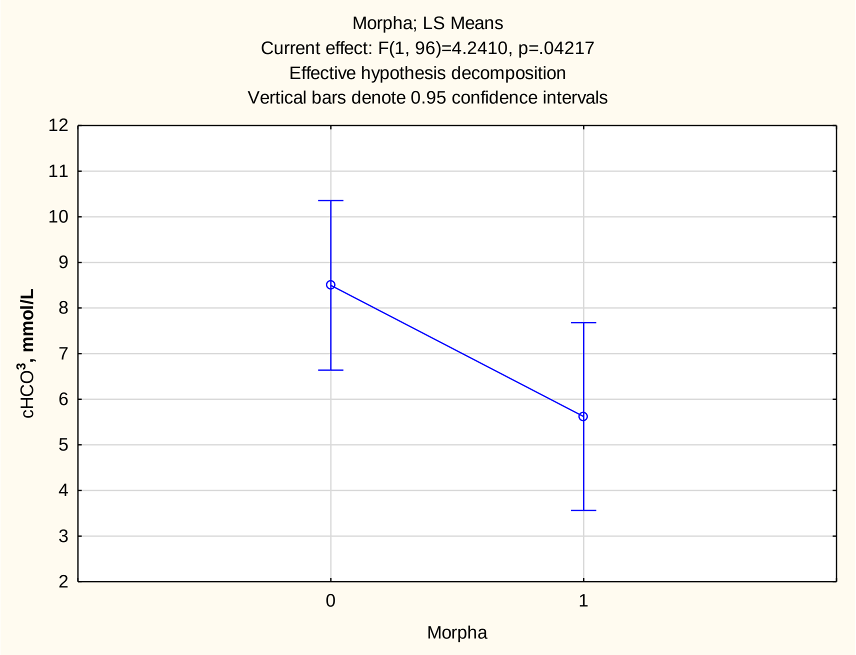

Figure 1: Difference in blood cHCO3 level in the studied samples

Figure (1) - shows the results of the one-way ANOVA, and there are statistically significant differences in the levels of cHCO3 in the blood of samples. And high cHCO3 in samples Maculatus(0) ($p=0.04 \leq 0.05$).

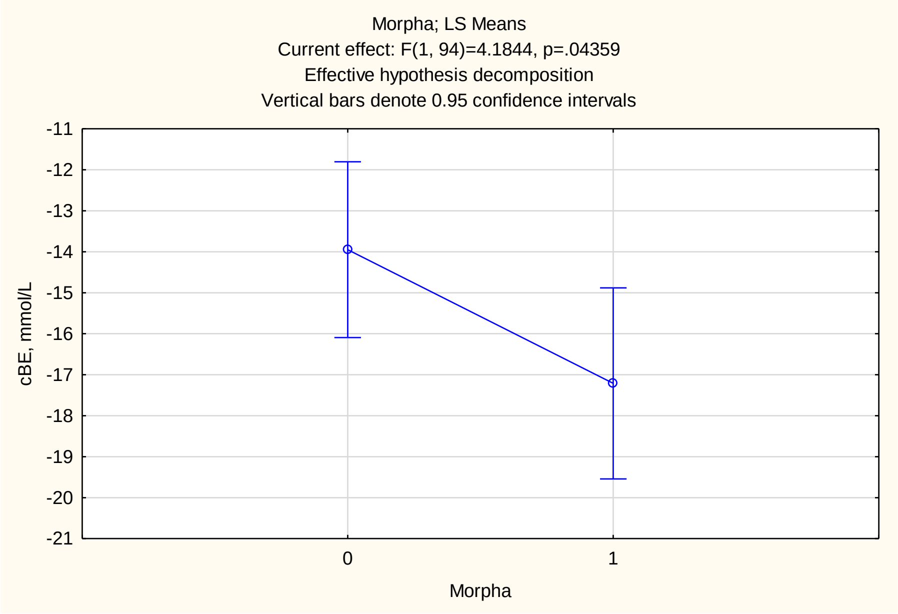

Figure 2: Difference in blood cBE level in the studied samples

Figure (2) - shows the results of the one-way ANOVA, and there are statistically significant differences in the levels of cBE in the blood of samples. And high cBE in samples in samples Maculatus(0) ($p=0.04 \leq 0.05$).

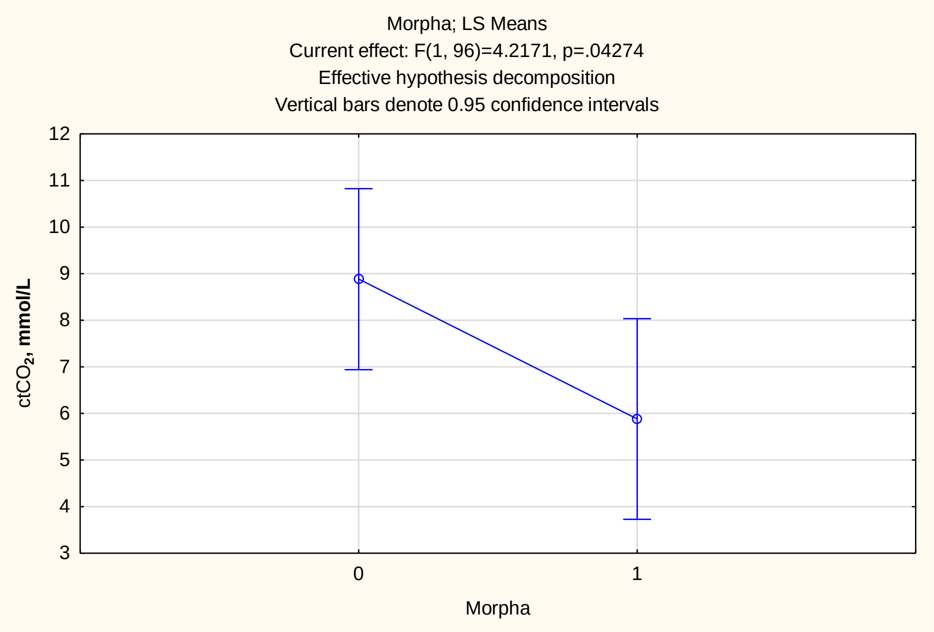

Figure 3: Difference in blood ctCO2 level in the studied samples
Figure (3) - shows the results of the one-way ANOVA, and there are statistically significant differences in the levels of ctCO2 in the blood of samples. And high ctCO2 in samples Maculatus(0) (p=0.04 ≤ 0.05).

The concentration of carbon dioxide - $ctCO_2$ is reduced in animals from the population from Lake Kurtan, respectively, the excess (or deficiency) of bases (cBE) in the blood is significantly higher, and the sum of bases of all blood buffer systems (cBB) is lower (Table 2). The content of sodium and potassium ions in the blood of animals from the population from the lake. Kurtan is significantly higher due to their high concentration in the environment. At the same time, the content of calcium ions is reduced in comparison with the non-saline territory, which is probably due to its excretion under conditions of alkalosis. High values of hematocrit (Hct = 35.4±1.9) were also noted there, which is associated with the features of the geochemistry of the environment, he high value of hematocrit in the population living in the territory of the biogeochemical province is associated with a change in osmotic pressure under salinity conditions.High mineralization of the habitat leads to excessive loss of fluid by the body, and, accordingly, a decrease in the volume of the liquid part of the blood. The calcium channel starts working. In the body of moor frogs from the population from the lake. Curtan begins to work transport to remove calcium and sodium (since the salinization of the environment). Therefore, the dynamics of these ions is positively correlated.

Ionized calcium Ca^{+2} plays a key role in the regulation of intracellular processes. It is a magnesium antagonist. The level of ionized calcium can change significantly with changes in the acid-base state of the blood: with alkalosis it decreases, with acidosis it increases. Tolerance to acid stress in amphibians depends on the flux of Ca^{+2} ions, and that different calcium channels are activated under different pH conditions. An influx of Ca^{+2} is essential for survival in acidic pH conditions. maintaining optimal intracellular pH is critical for organism homeostasis, but it can be influenced by various environmental conditions such as environmental salinity and acidity [12]. Indeed, an increase in the level of Ca^{+2} in water reduces the diffusion loss of ions.

The accumulation of bicarbonates in the body fluids of animals living in the Forest Park zone ($cHCO_3$=8.9±0.87) (Table 2) occurred mainly from internal buffer sources. It is assumed that the large efflux of bicarbonate from the animal is a consequence of the dissolution of $CaCO_3$ stores and delayed adaptation of bicarbonate retention mechanisms [13]. Our study showed that the indicated polymorphism (Maculatus, striatal) manifests itself at the level of blood physiology and affects such indicators as the content of bicarbonate ions, carbon dioxide and an excess (or deficiency) of bases). In striata, bicarbonate ion 5.6±1.04, base excess -8.5±9.9, and carbon dioxide 5.9±1.1 are less than in Maculatus. The lower concentration of carbon dioxide in the blood of animals of the striata morph determines the lower excess of bases and the level of bicarbonate ions, respectively (Table 3).

A decrease in skin permeability in individuals with a striatal morph leads to an increase in the role of pulmonary respiration, which, in turn, is associated with an increase in hematopoiesis (an increase in the number of erythrocytes and an amount of hemoglobin); this reflects the low efficiency of the potassium-sodium pump, which is responsible in amphibians for skin transport [14, 15].

4. Conclusions

High resistance to anthropogenic transformation of the environment is characteristic of *R. avalis*, which have such a genetic variant, as it can be said that individuals with a dorsomedial stripe, having hereditary physiological features that are unlikely to be adaptive under normal conditions, receive selective advantages in territories with a changed environmental chemistry. Thus, population polymorphism is a universal strategy that ensures the preservation of the integrity of the species based on the constant interaction of hereditary variability, random gene drift and natural selection in a normally fluctuating environment.

References

[1] B.A. Pierce. (1993). The effects of acid precipitation on amphibians. Ecotoxicology. 2: 65-77.

[2] R.B. Shartau, D.W. Baker, D.A. Crossley, C.J. Brauner. (2016). Preferential intracellular pH regulation: hypotheses and perspectives. Journal of Experimental Biology. 219(15): 2235-2244.

[3] E. Sils. (2010). Population characteristics of hematological parameters of the peripheral blood of postmetamorphic Rana ridibunda pall. In urbanized areas. Russian journal of ecology. 41(2): 189.

[4] W. Burggren, N. Bautista. (2019). Invited review: Development of acid-base regulation in vertebrates. Comp Biochem Physiol A Mol Integr Physiol. 236: 110518.

[5] R.M. Heuer, M. Grosell. (2014). Physiological impacts of elevated carbon dioxide and ocean acidification on fish. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 307(9): R1061-R1084.

[6] B.M. Koeppen. (2009). The kidney and acid-base regulation. Adv Physiol Educ. 33(4): 275-81.

[7] N. Heisler. (2004). Buffering and H+ ion dynamics in muscle tissues. Respiratory physiology & neurobiology. 144(2-3): 161-172.

[8] R. Occhipinti, W.F. Boron. (2015). Mathematical modeling of acid-base physiology. Prog Biophys Mol Biol. 117(1): 43-58.

[9] D. Glandt. (2006). Der Moorfrosch: Einheit und Viefalt einer Braunfroschart; 11 Tabellen. Laurenti: pp.

[10] D. Dolmen, J.K. Skei, I. Blakar. (2008). Scandinavian amphibians: their aquatic habitat and tolerance. Fauna norvegica. 26.

[11] R.A. Byrne, T.H. Dietz. (1997). Ion transport and acid–base balance in freshwater bivalves. Journal of Experimental Biology. 200(3): 457-465.

[12] S. Perry, K. Gilmour. (2006). Acid–base balance and CO2 excretion in fish: unanswered questions and emerging models. Respiratory physiology & neurobiology. 154(1-2): 199-215.

[13] M. Busk, E.H. Larsen, F.B. Jensen. (1997). Acid-base regulation in tadpoles of Rana catesbeiana exposed to environmental hypercapnia. The Journal of experimental biology. 200(19): 2507-2512.

[14] S.Y.L. Vershinin V.L. (2001). Specificity Of Erythropoiesis And Lymphopoiesis Of The Striata Morph In Underyearlings Of The Year Rana Arvalis Nilss. And R. Ridibunda L. In An Urbanized Area // Structure And Functional Role Of The Animal Population In Natural And Transformed Ecosystems. . . Dnepropet Rovsk: Dnepropetrov. Nat. Un-T. With. 123 124.

[15] V. Vershinin. (2008). Morpha striata in the members of the genus Rana (Amphibia, Anura), the reasons of adaptability to environmental changes. Zhurnal Obshchei Biologii. 69(1): 65-71.