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Parameters of the Transmembrane Transport of *Pelophylax ridibundus* in the Conditions of Thermal Reservoirs

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Abstract

Study of *Pelophylax ridibundus* skin physiological parameters in a thermal basin shows that there are seasonal differences in PD (potential difference) and I_{sc} (active transportation parameter). Amphibians studied in the summer time in a control area showed some differences compared to specimens caught in summer time in a basin with higher temperatures (River Malaya Kushva). We may suppose that this is connected with the habitation adaptation of the population.

Keywords: *Pelophylax ridibundus*, marsh frog, thermal pools, Ussing chamber, skin permeability, short-circuit current.

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1. Introduction

Pelophylax ridibundus are from the complex of European green frogs with a semiaquatic way of life. This is a species with a high tolerance for high concentrations of salts dissolved in the water: moreover, it is resistant to high temperatures [1]. It is known that cold-blooded animal cell and tissue resistance to high temperatures may be considered an adaptation among species and populations to ambient influence [2].

Tissue heat resistance of this species has been frequently studied [3]. Studies of geographical variability among the lake frog (*P. ridibundus*) have found differences in this value among "European" and "Asian" forms of this species. Due to the aforementioned characteristics (the unique combination of heat resistance among lake frogs and the multifunctionality of their skin), the purpose of this paper is to study and comparatively analyse the specific characteristic of transmembrane transportation in the skin of lake frogs living in a cooling basin (River Malaya Kushva, NTMK plant) and normal conditions. It also seeks to analyse skin transportation parameters depending on various ecological conditions.

Studies of the population-specific characteristics of the physiological parameters of amphibian skin using an Ussing chamber system are very few: this is why the present research is up-to-date and new. The **research purpose** is a comparative analysis of the specific characteristics of transmembrane transportation in the skin of lake frogs living in cooling basins and in normal conditions.

2. Methods

The selection of animals for the experiment was conducted by hand from natural populations during their active life cycle phase. The materials were collected in the city of Nizhny Tagil, Sverdlovsk region, in River Malaya Kushva, which flows through a production site of the Nizhnetagilsky metallurgical plant (founded in the 1970s). Waste waters from this plant are discharged into the upper part of the in order to cool and precipitate plant waste water discharges. The material in River Malaya Kushva river was collected in early spring (March) and summer (August) in 2015-2016. 38 specimens were caught over 2 winter periods, and 29 in summer. The total number of caught and studied specimens is 68. In Kalinovskye razrezy forest park (the control water reservoir) in 2015, we caught and studied 21 specimens.

The water temperature in the researched basins was measured with the help of an aquarial thermometer with an external temperature sensor and a scale interval of $0.1C^0$. In spring, the water temperature reached +24.7 C, and ambient temperature did not exceed -18 C. In summer time, the water temperature reached +34.6 C.

For the experiments, we used the skin pieces of 89 individuals. In the Ussing chamber, silver chloride electrodes (AgCl) filled with NaCl – agar bridges were placed before the experiment. We used belly skin (ventricular piece – "Belly") and back skin (dorsal piece – "Back"). The pieces were cut one by one and then placed into the Ussing chamber "World Precision Instruments, Inc" (Germany) an with internal hole diameter 6 mm. The chamber was filled with Ringer's solution for cold blood, containing (mm): NaCl – 8.6g, KCl – 0.3g CaCl – 0.25g. The tests were conducted at room temperature (22-23C). Using the Ussing chamber system, we recorded the following values for each skin piece – I_{sc} (short circuit current), PD (potential difference). Furthermore, the data was re-calculated according to the following formula:

2.1. Short circuit current

$$I_{sc}(\mu A/\text{cm}^2) = I(s)/\pi R^2 \text{ (chamber diameter)} \quad (1)$$

The calculated values were processed by Statistica 6.0 and MS Excel. The comparison of values between the dorsal and ventricular pieces was based on the Student criterium. Interspecific differences were assessed using ANOVA.

3. Results

In the initial research stage, we assessed the volt and ampere characteristics of each skin piece (ventral and dorsal) for each specimen. We compared the ventral and dorsal PD and Isc for each sample. This analysis (table 1) showed the difference between the ventral and dorsal pieces of amphibians caught in the summer time in River Malaya Kushva (p=0.05). It is noted that specimens caught in N. Tagil in cold periods are characterized by similar conductivity in the belly and back pieces, in contrast to the samples caught in summer period. This may be linked to the abnormal thermal conditions in River Malaya Kushva.

Table 1: Comparison of ventral and dorsal pieces from various locations and seasons.

Parameter	PD(mV)		I_{sc}(mA/cm^2)	
Location of the skin flap	Belly*	Back*	Belly	Back
Kushva (March)	-12±9.2	-9.6±8.5	17.9±9.69	17.4±13.4
Kushva (August)	-30.4±16.2**	– 13.8±10.1**	38.71±11.58$^{\Delta}$	32.26±11.02 $^{\Delta}$
Control	12.01±13.4	– 9.5±3.9	16.73±12.1	12.54±2.43
*Belly – dorsal flap of skin				
*Back – ventral flap of skin				
** - significant differences (p<0.05)				
Δ - significant differences (p<0.05)				

The analysis of the "short circuit current" - I_{sc}(mA) – of the dorsal and ventral pieces in the studied samples registered maximum values among specimens caught in the summer period in the basin with higher temperature conditions. We note that only this sample has reliable differences between the dorsal and ventral skin pieces (p<0.000). Animals caught in the spring at Kalinovskye razrezy (the control water reservoir) have no differences between the dorsal and ventral pieces.

The "short circuit current" (Fig. 1) data show reliable differences among the studied samples. So, the ventral and dorsal pieces I_{sc} of amphibians caught in summer in N. Tagil reliably differ from all the other samples (p=0.95). We note the similarity of I_{sc} in animals from the forest park and River Malaya Kushva (caught in spring). The data is 95% similar for ventral pieces and 45% similar for dorsal pieces.

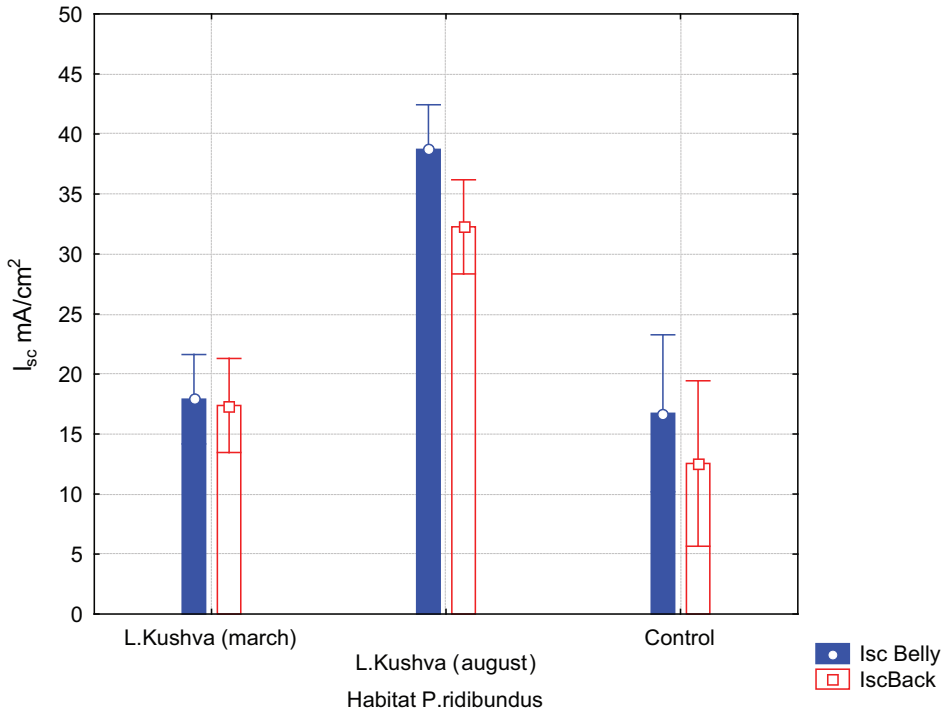

Figure 1: Comparison of the "short-circuit current" (I_{sc} mA/cm^2) in the investigated samples.

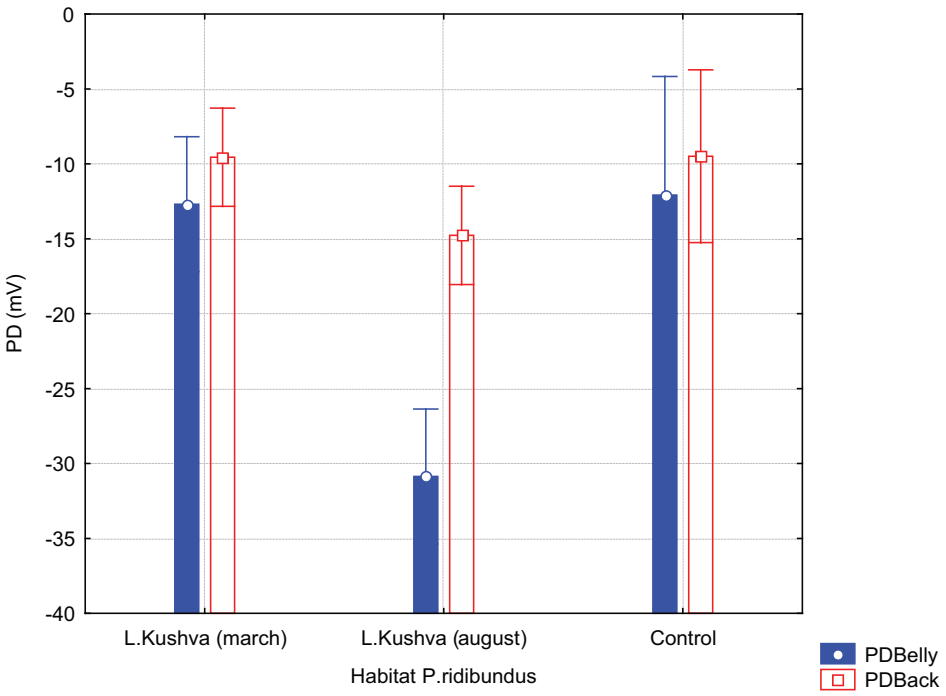

Figure 2: Comparison of potential difference (PD mV) in the investigated samples.

The comparative results of PD (mV) for dorsal and ventral pieces of the various samples are shown in Fig. 2. Minimal PD values are registered among amphibians caught in

the summer in River Malaya Kushva. The average PD value of a ventral piece is -20.2 ±6.8mV, while for a dorsal piece it is -20.8±10.2mV. An almost complete match of PD values is noted in samples from the forest park and River Malaya Kushva. The analysis showed similar potential difference values among animals from the forest park and River Malaya Kushva (the data is 99% alike). It is important,that active transportation differences in frog skin are found in the ventral pieces, as it is the belly skin that mainly participates in active exchange with the environment [5].

4. Conclusion

We have assessed such skin transportation characteristic in terms of PD and I_{sc} for the ventral and dorsal pieces of each *Pelophylax ridibundus* sample. A comparative analysis showed reliable differences between the physiological characteristics of amphibian belly and back skin only among the samples caught in the summer time (River Malaya Kushva, August).

There are differences in skin PD and I_{sc} values between the samples caught in the summer in River Malaya Kushva and the other two. There are no reliable differences found between samples caught in the winter period in River Malaya Kushva and Kalinovskye razrezy.It is probable that the data on amphibian skin electrophysiology reflects an abnormal operating mode for the sodium channels of animals living in the thermal basin or a labile switch in their operation.

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