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Physiological Parameters of Amphibians in Ecosystems of Urbanized Territories

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Abstract—An example of *Rana arvalis* Nilss. was used to demonstrate specific features of the metabolism, neuromuscular system, and skin permeability for sodium of amphibians from areas with different levels of urbanization and environmental pollution. The difference in skin permeability between striated and nonstriated morphs was revealed for the first time. Some regularities revealed in the study indicate that parameters characterizing the physiological state of amphibians can be used for more complete and unbiased assessment of their populations and the extent of changes occurring in them.

INTRODUCTION

Investigation of the physiological specificity of animals from populations exposed to extreme conditions of the anthropogenically transformed environment is the way to quickly obtain (in comparison with zoological methods) new data on the processes of adaptation and microevolution in populations inhabiting the corresponding landscapes. According to Shvarts (1973), the population determines its fate by controlling the physiological state of its constituent individuals, but this takes place only until these individuals remain in the integrated population as its members.

As aquatic organisms are very sensitive to prolonged action of low concentrations of pollutants, the state of aquatic biocenoses is, in the end, the best criterion of changes in water chemistry (Buyanovskaya, 1973). Many authors noted individual (and population-specific) responses of amphibians to pollution and urbanization (Pliss and Khudolei, 1979; Rose and Harshbarger, 1977; Ilosvay, 1977). The most informative parameters include normal and readily reversible structural and functional reactions characteristic of phenotypically stable organisms (including amphibians) with largely autonomous development (Schmalhausen, 1983). Shvarts (1954) noticed that morphophysiological characteristics of amphibians are highly sensitive to the contents of microelements in water.

MATERIALS AND METHODS

The present study is based on materials obtained by investigating natural *Rana arvalis* Nilss. populations widely distributed within the Ekaterinburg city limits. With respect to the level of anthropogenic transformation, we distinguished in this large industrial city four zones to which amphibian habitats are confined (Table 1). The basic criterion of zoning was the degree of housing development (number of stories, density of buildings, and other corresponding features, organization of the territory in general, and the level of pollution).

The functional state of the neuromuscular system was determined using an ESL-2 electronic stimulator, an accommodometer, and silver chloride electrodes with a screened chamber. Skin permeability for sodium was measured by means of two pairs of silver chloride electrodes with an agar electrolytic bridge. Oxygen consumption was determined using an AKTs-2 digital oxygen analyzer.

RESULTS AND DISCUSSION

Effect of Urban Environmental Pollution on Sodium Balance in Amphibians

Under conditions of considerable pollution, amphibians are known to demonstrate changes in protein and lipid metabolism and an increase in the mass of cell membranes, which may reflect their decreased permeability for pollutants (Misyura, 1989). The analysis of skin permeability for sodium in adult and juve-

Table 1. Sodium permeability of skin in adult and juvenile frogs, mV

Zone	Young of the year				Adults	
	REP	N	REP (with KJ)	N	REP	N
II	19.0 ± 5.8	15	19.9 ± 6.9	6	4.95 ± 2.05	24
III	30.8 ± 5.8	15	28.6 ± 4.9	12	6.88 ± 2.89	12
IV	42.7 ± 7.5	9	38.6 ± 5.7	9	12.47 ± 2.05	24
K	47.1 ± 4.6	24	30.9 ± 6.9	6	17.90 ± 2.89	12

Note: Here and below, II is the zone of multistory housing, III is the zone of low housing, IV is the park forest zone, and K is a suburban area.

Table 2. Oxygen consumption and liver index in juvenile and adult *R. arvalis* frogs

Zone	O_2 consumption, ml		Average liver index, ‰			
	young of the year	*N*	young of the year	*N*	adults	*N*
EURT	0.000676 ± 0.000019	14	53.05 ± 2.0	30	34.6 ± 5.6	11
II	0.001551 ± 0.000023	10	51.4 ± 0.54	651	104.3 ± 7.5	5
III	0.001589 ± 0.000032	5	53.5 ± 0.79	306	54.8 ± 5.8	8
IV	0.002299 ± 0.000028	7	53.07 ± 0.38	1350	56.7 ± 5.5	9
K	0.000941 ± 0.000022	11	48.9 ± 0.53	666	43.1 ± 1.5	117

nile (young of the year) *R. arvalis* revealed some significant differences in this parameter (Table 1). Skin permeability in frogs from the zones of multistory housing and low buildings was more than twice as low as in those from the park forest zone and the suburban population. In general, it decreased in inverse proportion to the pollution gradient. The significance of this trend, obtained in 1993 for juvenile *R. arvalis* and *R. temporaria*, was very high ($F = 8.432$, $p < 0.0001$). Interspecific differences were less significant ($p = 0.002$). On the whole, however, skin permeability for sodium in *R. temporaria* was lower than in *R. arvalis*. According to the data obtained in 1994, differences in skin permeability in *R. arvalis* young of the year were also significant ($F = 5.393$, $p = 0.002$). Upon treating the skin flap with KJ, REP values in juveniles from zone II remained virtually unchanged, whereas in animals from other zones they decreased, and, hence, significant differences between zones disappeared. After skin flap washing with Ringer solution, the difference did not appear again: the REP in juveniles from zone II remained at the same level, and in those from other zones it did not increase to the initial value.

The analysis of experimental data showed that morphs of *R. arvalis* significantly differ in skin permeability: in striated frogs, this parameter was significantly lower (under different testing conditions, significance of differences varied considerably, with *p* values changing from 0.004 to 0.0005). It was found that zonal differences in skin permeability resulted solely from an increase in the proportion of striated individuals in populations from zones II and III. Therefore, in this case we are apparently dealing with preadaptation, which is an important condition allowing animals to make the first step toward colonization of a new environment (Shvarts, 1980). Greater proportions of striated *R. arvalis* frogs in urban populations is a marker of changes in their genetic structure under new environmental conditions. Characteristics of skin permeability explain how the *striata* morph gained selective advantage in populations inhabiting anrthropogenically transformed territories.

Oxygen Consumption by Amphibians

Our results showed that oxygen consumption by mature male *R. arvalis* significantly varied from population to population and differed from that in the control group. Compared to control values, oxygen consumption by frogs from different zones was higher: in zone IV, by 17.3%; in zone III, by a factor of 2; and in zone II, by 72.4% (data statistically significant) (Vershinin and Tereshin, 1992).

Experiments on 14 animals captured in 1993 and 1994 in the 500–1000-Ci zone of the Eastern Ural radioactive trace (EURT) showed that the level of oxygen consumption by adult *R. arvalis* depends both on body weight and on the location of home range, but the former dependence is more significant ($F = 8.832$, $p < 0.0001$) than the latter (zonal) one ($F = 4.987$, $p = 0.006$). As the degree of urbanization increases (Table 2), oxygen consumption initially increases as well (from the control to zone III) but then decreases (in populations of zone II), which may be evidence for adaptive modifications of higher level than energy-consuming physiological ones (Shvarts, 1980). In the sample from the EURT territory, a considerable decrease in this parameter is noted, which we attributed to specific features of populations living under conditions of radioactive contamination: it was shown that exposure to radionuclide contamination may lead to a sharp inhibition of metabolic processes—hypooxygeny (Testov, 1993).

Using traditional zoological methods, it was found that large energy-consuming individuals and those with a hereditary high level of metabolism (Dobrinskii and Malafeev, 1974; Vershinin, 1995) prevail in amphibian populations living within city limits, i.e., in the area with the maximum level of anthropogenic impact. Comparison of changes in the level of oxygen consumption and the liver index in young of the year and adult frogs depending on the degree of urbanization demonstrated that the zonal dynamics of these parameters is similar. This is evidence that the same changes occur in populations of urbanized territories (Table 2).

Thus, frogs from the populations exposed to anthropogenic impact proved to have a higher level of metabolism. This fact suggests that the parameter of oxygen consumption by amphibians, combined with other biophysical (Tereshin and Vershinin, 1989) and electrophysiological tests (Vershinin and Tereshin, 1992), may be used in the general complex of bioindication methods, in particular, for estimating the degree of adaptive

Table 3. Excitability of nerve and muscle tissues of frogs

Zone	Nerve				Muscle				Heart index in juveniles	
	$V_0 \pm m$	N	$V_1 \pm m$	N	$V_0 \pm m$	N	$V_1 \pm m$	N	Cor, ‰	N
II	0.579 ± 0.059	11	0.454 ± 0.78	9	2.67 ± 0.83	12	3.87 ± 1.68	12	3.38 ± 0.04	708
III	0.433 ± 0.069	8	0.352 ± 0.88	7	2.42 ± 1.01	8	3.12 ± 2.06	8	3.16 ± 0.06	306
IV	0.457 ± 0.054	13	0.546 ± 0.78	9	1.72 ± 0.80	13	1.41 ± 1.76	11	3.03 ± 0.028	1376
K	0.383 ± 0.038	27	1.11 ± 0.46	26	2.26 ± 0.53	30	4.06 ± 1.08	29	2.88 ± 0.039	722

Note: V_0 and V_1 refer to excitability before and after washing, respectively.

shifts in populations inhabiting anthropogenic landscapes.

Specific Functional Features of Excitable Tissues

In the course of environmental transformation, animals specifically respond to changes in ambient conditions. At the same time, individuals with more perfect morphophysiological reactions are selected, and the population acquires heritable morphophysiological traits. This pathway is not advantageous thermodynamically. The next stage is selection of individuals capable of maintaining an energy balance without developing pronounced morphophysiological adaptations (Shvarts, 1980). The level of adaptation determines the profoundness of transformations in the population. This explains attention to the analysis of variation in the functional state of excitable tissues of the organism, i.e., of the nerve and muscle tissues determining the mobility of individuals, the rate of their reactions to external factors, and eventually the state of organism on the whole and probably the stability of a species under new conditions.

One of the integral parameters characterizing the functional state of excitable tissues, along with excitability, is the capacity for accommodation (Khodorov, 1969). This parameter reflects the level of functional lability of the tissue and directly depends on the accommodation capacity of individual nerve and muscle fibers (Bretag and Stampfl, 1975).

Accommodometry proved to be informative in the comparative study on estimating physiological activity of sapropel components (Tereshin *et al.*, 1981), i.e., this method detected changes in concentrations of microelements in the environment.

We analyzed specific features of excitability and accommodation capacity in *R. arvalis* frogs exposed to different levels of pollution and urbanization, and the relationships of these parameters with some characteristics of frog populations. Experiments were performed on 18 mature males during the field season of 1987–1988. The frogs were sampled from populations inhabiting different zones of urban landscape distinguished with respect to the level of housing development, pollution, and general exploitation of the territory by people (Vershinin, 1995).

Table 3 shows the results of determining excitability of the nerve and muscle tissues of frogs from these zones. According to the results of a two-way ANOVA, excitability of the nerve tissue had a significant zonal specificity (p = 0.05, F = 2.632), which disappeared after 30-min washing of the tissue in Ringer solution. A significant decrease in this parameter (i.e., higher excitability threshold), compared to that in the control, was observed as the level of anthropogenic impact and pollution increased.

The muscle tissue excitability had certain specific features but, as significance of differences was low, we can only note a certain tendency.

Lower excitability (higher excitability threshold) was revealed in animals from zones II and III, where they are exposed to the greatest anthropogenic impact. The decrease in excitability may be regarded as a sign of adaptation of physiological systems to new environmental conditions. It should be noted that after washing the preparations for 30 min in fresh Ringer solution, the values of excitability of the muscle tissue decreased (excitability thresholds became higher) in frogs from all investigated zones (except zone IV), not differing significantly between themselves. This fact suggests that changes in muscle excitability in this case are not irreversible.

Thus, changes of muscle excitability in frogs from territories with different levels of urbanization differ from corresponding changes in the nerve tissue. The greater the anthropogenic transformation of the environment, the lower the excitability of nerve tissue (the excitability threshold for rectangular electric pulses progressively increases). The values of absolute excitability of the amphibian nerve tissue may serve as a criterion for estimating the level of anthropogenic impact on the population and the ecosystem.

The results of determining the accommodation capacity of nerve and muscle tissues in frogs from populations living within city limits are shown in Table 4. The accommodation constant of the nerve tissue had significant zonal differences ($p < 0.05$, $F = 3.018$). Its values in frogs from zones III and IV were lower than

Table 4. Accommodation capacity of nerve and muscle tissues of frogs

Nerve	Muscle				Zone			
	$L_1 \pm m$ ($T = 0.25$)	N	$L_2 \pm m$ ($T = 2.5$)	N	$L_1 \pm m$ ($T = 2.5$)	N	$L_2 \pm m$ ($T = 2.5$)	N
II	5.00 ± 0.64	10	34.71 ± 4.75	9	0.529 ± 0.36	12	4.99 ± 0.91	12
III	3.93 ± 0.69	8	14.90 ± 5.41	7	0.555 ± 0.44	8	5.64 ± 1.11	8
IV	3.49 ± 0.58	12	20.41 ± 4.75	9	0.726 ± 0.34	13	6.74 ± 0.88	13
K	5.39 ± 0.38	27	22.25 ± 2.79	26	0.814 ± 0.22	30	5.00 ± 0.59	28

Note: L_1 and L_2 refer to accommodation constant before and after washing, respectively.

in frogs from zone II and the control. After 30-min washing in fresh Ringer solution, nerve tissue preparations retained this zonal specificity ($p < 0.05$, $F = 2.894$). Accommodation constants were noticeably reduced in zones III and IV, compared to those in zone II and the control.

The highest accommodation capacity of the nerve tissue (the lowest accommodation constants) was revealed in animals from zones III and IV. The same is true of the dynamics of the accommodation constant. It may be assumed that frogs suffering from relatively low (zone IV) and moderate (zone III) anthropogenic impact are characterized by a comparatively greater adaptability of the nervous system.

The pattern of changes in the constant of muscle tissue accommodation depending on the level of urbanization differs from that of nerve tissue accommodation (Table 4). The lowest constant (the highest accommodation capacity) for muscle tissue was found in frogs from zone II. Accommodation capacity decreased (accommodation constant increased) at lower levels of anthropogenic impact. After 30-min washing of muscle tissue in fresh Ringer solution, this capacity became slightly lower in frogs of the control group than in frogs from other zones, although the difference was nonsignificant. These results demonstrate that frogs from populations exposed to the strongest impact (zones II and III) are characterized by relatively higher adaptability than frogs from the control group and zone IV.

The accommodation capacity of excitable tissues in frogs from the urban territories was also studied in a stronger gradient of the test stimulus ($T = 2.5$ m s). The accommodation constants for nerve and muscle tissues of frogs from all the investigated zones did not differ significantly from those in the control group. The same was observed after 30-min washing in fresh Ringer solution.

Thus, frogs from zones II and III proved to have lower nerve excitability (a higher excitation threshold) than control animals: 0.579–0.433 vs. 0.457–0.383 m s. This may be associated with an increase in the effect of disturbance factor and a high locomotor activity of larvae and juveniles in such populations. In larvae, hyperactivity is conditioned, *inter alia*, by the action of pollutants (Taylor *et al.*, 1990), which is indirectly confirmed by a high heart index in juvenile frogs from such zones (Vershinin, 1992). The patterns of changes in the excitation threshold and the heart index are very similar (Table 3). The observed differences in excitability of nerve tissue and the excitation threshold indicate that, in addition to previously revealed adaptive changes, the frog populations under study are characterized by physiological adaptation, as these differences leveled off after tissue washing in Ringer solution.

The constant of nerve tissue accommodation and its dynamics in animals from zones III and IV were significantly lower than in zone II and the control group (3.49 and 3.92 vs. 5.0 and 5.38 ms, respectively; $p = 0.038$, $F = 3.018$). This is evidence for the highest accommodation capacity of these frogs, i.e., for the existence of adaptive physiological changes in the absence of significant population differences in zones where anthropogenic pressure did not reach maximum values. Frogs from the suburban population apparently have a lower accommodation capacity, living beyond the zone of strong anthropogenic impact, and animals from zone II demonstrate some profound qualitative differences at the population level (Vershinin, 1995), which makes physiological adaptations less important. Physiological compensations as a way of adaptation to different environmental conditions are very elaborate but thermodynamically inefficient (Shvarts, 1980). Apparently, excitability of the nerve tissue reflects the levels of disturbance and pollution under conditions of urbanization, while its accommodation capacity reflects the degree of adaptive changes in populations living in the anthropogenically transformed environment.

Our investigation revealed some physiological adaptations in populations of *R. arvalis* living within city limits and provided the possibility of more detailed and thorough evaluation of previous results obtained in the same populations by zoological methods. The appropriate combination of different methods is a promising approach to obtaining adequate and comprehensive information on processes occurring at different structural levels of the biota. This should allow researchers to reveal general regularities of the investigated processes and to estimate the extent and direction of the observed changes.

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