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HEMATOLOGICAL INDEXES OF *Rana ridibunda* IN CLEAN AND CONTAMINATED PONDS

T. Y. Peskova¹ and T. I. Zhukova¹

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INTRODUCTION

Experimental research has been performed of the influence of pesticides on blood of some species of anurans (Gromysz-Kalkowska and Szubartowska, 1986; Szubartowska, 1990; Zhukova and Peskova, 1996). We had determined in laboratory experiments that the blood reaction of *Rana ridibunda* on pesticide pollution depends on dose of toxicant and duration of its influence (Zhukova and Peskova, 1999). In this paper there are some results of our study of hematological indexes of *Rana ridibunda* living in natural ponds with different degree of pesticide pollution.

MATERIAL AND METHODS

The study was conducted in 1998 – 1999 in the Kuban Region (Northern Caucasus). The object is *Rana ridibunda* (200 individuals) from the rice field contaminated by pesticides and a clean pond in arboretum of Kuban State

¹ Kuban State University, Stavropolskaya St., 224/1-49, 350040 Krasnodar, Russia; E-mail: peskova@kubannet.ru.

TABLE 1. The Contents of Residual Chlororganic Compounds (COC) in Water (mg/liter), Ground (mg/kg), and tissues of *Rana ridibunda* (mg/kg)

Object of research	γ -HCCH	DDT	DDD	DDE	Σ COC
Rice field					
Water	N.S.	0.0030	N.S.	0.003	0.0033
Ground	0.0281	0.0343	0.0100	N.S.	0.0724
<i>Rana ridibunda</i>					
muscles	N.S.	0.0027	0.0034	N.S.	0.0061
liver	0.0296	0.0400	0.0229	N.S.	0.0925
Pond in arboretum					
Water	0.0003	0.0006	0.0001	N.S.	0.0010
Ground	0.0030	0.0038	N.S.	N.S.	0.0068
<i>Rana ridibunda</i>					
muscles	0.0003	0.0025	N.S.	N.S.	0.0028
liver	0.0012	0.0048	N.S.	N.S.	0.0060

N.S., not significant.

University. Residual quantities of chlororganic and phosphororganic compounds in water and ground of these ponds as well as in tissues of *Rana ridibunda* are estimated using the method of gasliquid chromatography in the Laboratory of Water Toxicology (KrasSRIFI).

RESULTS AND DISCUSSION

The differences in total chlororganic compounds (COC) in water of rice field and pond of arboretum are connected to quantity of DDT (it is 5 times higher in water of rice field); remaining components (HCCH and metabolites of DDT) are about identical in water of both ponds (Table 1).

The sum of COC in ground of rice field is 10 times higher, than in a pond from arboretum (difference are marked for HCCH, DDT and DDD; only DDE is found in not significant quantities in both ponds). The contamination of ponds promotes accumulation of chlororganic compounds in tissues of *Rana ridibunda*. The maximum concentrations of toxicants are found in a liver. It is equal to the level of concentrations in ground of both ponds and higher, than in water (6 times in arboretum and 28 times in rice field).

The maximum levels of erythrocytes and hemoglobin are shown for frogs in both ponds in summer; in spring and in autumn these indexes significantly declined. The level of leukocytes for frogs from the rice field in summer and

TABLE 2. Hematological Indexes of Adult Males of *Rana ridibunda* from Clean and Contaminated Ponds of Northern Caucasus, in Different Seasons of Researches ($X \pm m$)

Time of research	Pond	Erythrocytes, thousand per mm ³	Hemoglobin, g %	Leukocytes, thousand per mm ³
Spring	clean	290 \pm 10.9	8.1 \pm 0.48	11.0 \pm 2.1
	contaminated	340 \pm 12.0	8.9 \pm 0.77	13.4 \pm 1.9
Summer	clean	520 \pm 13.1	11.5 \pm 0.71	12.1 \pm 2.0
	contaminated	650 \pm 12.2	14.2 \pm 0.82	22.5 \pm 2.1
Autumn	clean	260 \pm 10.5	7.9 \pm 0.52	14.7 \pm 1.9
	contaminated	295 \pm 10.1	8.2 \pm 0.47	19.8 \pm 2.1

TABLE 3. Hematological Indexes of *Rana ridibunda* from Clean and Contaminated Ponds of Northern Caucasus in Summer

Age	Pond	Erythrocytes, thousand per mm ³		Hemoglobin, g %		Reticulocytes, %	
		males	females	males	females	males	females
Adults	clean	520 ± 13.1	550 ± 14.0	11.5 ± 0.71	12.8 ± 0.73	0.3 ± 0.05	0.7 ± 0.06*
	contaminated	650 ± 12.2	695 ± 14.2*	14.2 ± 0.82	16.5 ± 0.91	1.3 ± 0.07	1.6 ± 0.06*
Juveniles	clean	390 ± 11.2		7.5 ± 0.65		1.8 ± 0.08	
	contaminated	490 ± 12.0		9.7 ± 0.70		4.1 ± 0.10	

* The differences are significant in comparison with the males ($P = 0.05$).

autumn is authentically higher, than this index in spring (Table 2).

We have conducted further analysis of hematological indexes of *Rana ridibunda* in summer taking into account sex and age of animals (Table 3). We had noted sexual dimorphism in number of erythrocytes in the contaminated pond, and also in number of reticulocytes (both in contaminated pond, and in a clean pond); in all cases these indexes are authentically higher for females.

The number of erythrocytes and hemoglobin in juveniles is authentically less than in adults. The quota of reticulocytes in juveniles is more (2.8 – 3.6 times), than in adults, thus the hemopoiesis was more intensive in juveniles.

All hematological indexes of *Rana ridibunda* (both adults and juveniles) in summer are authentically higher (by 21 – 29%) in contaminated pond. The number of reticulocytes significantly increases in contaminated pond 2.1 – 4.3 times. Similar changes were marked earlier (Zhukova and Fic, 1996). However absolute values of these indexes in our research are authentically higher, than it is known from the literature. It is possible to suspect that it is connected with the duration of habitation of amphibians under conditions of pesticide contamination.

The number of leukocytes of *Rana ridibunda* from the rice field is authentically higher (1.9 times), than of amphibians from a pond, that testifies to an increase of protective function of a blood. The number of neutrophils is more in the leukocytic formula of frogs' blood in contaminated ponds.

There is a sexual dimorphism of index of heart in *Rana ridibunda*: in spring (in spawning period) it is higher in females. The index of heart is higher in frogs from

the rice field than from the pond: in adult animals are 7.9 ± 0.66 and $5.0 \pm 0.71\%$, in juveniles are 9.6 ± 0.72 and $6.0 \pm 0.55\%$. The increase of index of heart testifies to higher level of metabolism of amphibians.

The picture of red blood of amphibians permanently inhabiting ponds with pesticide pollution (DDT and its metabolites) is like the one under the short duration of small doses of pesticides (Zhukova and Peskova, 1999). The increase of oxygen capacity of amphibian's blood in the presence of pesticides is long-term adaptation; it is manifestation of effect of directional selection.

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