Russian Journal of Ecology, Vol. 27, No. 5, 1996, pp. 361–365. Translated from Ekologiya, Vol. 27, No. 5, 1996, pp. 378–382.

Utilization of Amphibians in Bioindication Research on Territories of the Eastern Urals Radioactive Trace

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Received September 6, 1995

Abstract—An investigation of a population of *Rana arvalis* Nilss. carried out in 1993 in a region of Lake Berdanish on territory contaminated by radionuclides revealed changes in the population structure and the appearance of specific traits and physiological and genetic distinctions from natural populations. These distinctions can be used for bioindication and the study of microevolutionary processes under new environmental conditions.

Recently, in world nature protection, there has been a noticeable increase in the number of studies employing biological methods to estimate the condition of aquatic and terrestrial ecosystems. The simplicity and high sensitivity of most biotests provide information that cannot be obtained by traditional chemical analyses and traditional equipment. Biological methods allow both the determination of the extent and intensity of the influence of a pollutant and tracking of the dynamics of ecosystemic degradation over time and space. Analysis of the literature (Zhukova and Kubantsev, 1980) and our data (Pyastolova *et al.*, 1990; Pyastolova and Danilova, 1986; Vershinin, 1982, 1990) reveals that the responses of animals to various doses of pollutants are different. Therefore, it is necessary to take into account the biological characteristics of the groups of animals used, the norm of response, their ontogenetic characteristics, and their place and role in the structure of the biogeocenoses under study in investigations of the influence of anthropogenic factors on biological objects.

The appearance of such a factor as ionizing radiation leads not only to changes in the structure of communities, but also to the origination of forms with new properties, essentially serving to accelerate the rate of evolution. For some species, radiation is pernicious and leads to their elimination; others turn out to be stable, and their role and even numbers in a community can increase. Ionizing radiation decreases the viability of animals and may result in infection by endo- and ectoparasites (Lebedinskii and Ryzhkova, 1994) and favorable conditions for the spread of transmissive and other native-nidus diseases (Krivolutskii, 1983; Krivolutskii *et al.*, 1993; Il'enko and Krapivko, 1993).

The investigation of vertebrates—objects having a structural plan like that of humans, and similar physiological, biochemical, genetic and other parameters—is extraordinarily important in studying adaption to ionizing radiation (Il'enko, 1974; Il'enko *et al.*, 1974). However, most investigations under natural and experimental conditions were carried out on mammals (rodents and the laboratory animals) and less frequently on amphibians (Usachev *et al.*, 1993; Cherdantsev *et al.*, 1993). Because of their biological peculiarities, amphibians are very convenient for monitoring ecosystems (both terrestrial and aquatic). Their wide distribution, clear population borders; polymorphism; ability to accumulate pollutants, heavy metals, and radionuclides; possibility for use under laboratory conditions; and well-studied ecology and biology allow us to use this group of vertebrates as bioindicators.

MATERIALS AND METHODS

Today, considerable attention is given to the investigation of the ecological situation on the territory of the Eastern Urals Radioactive Trace and the study of the long-term action of radionuclides on organisms under natural conditions. The present work was carried out on the territory of the Experimental Research Station at the Mayak Industrial Association (Chelyabinskaya oblast). As a testing ground, a territory contaminated with radionuclides was used near Lake Berdanish, outside the immediate influence of other types of pollution. The control lot was in a region of the Dolgobrodskoe reservoir, where the influence of all pollution was excluded. The frog *Rana arvalis* Nilss. was used as the object of investigation, as it is widespread in this region. This work was fulfilled in the spring–summer period of 1993.

The state of populations was estimated by using the morphological and morphophysiological parameters of the animals that emerged in the current year and adults, the occurrence of various anomalies, and reproductive capacity (the number of eggs per clutch, egg diameter, traits of juveniles, and the survival of embryos and larvae).

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RESULTS AND DISCUSSION

To investigate reproductive parameters, 19 experimental and 12 control egg masses were taken, their volume was measured, and the number of eggs per clutch was determined. The differences in the number of eggs were insignificant, and, on average, there were 896.3 ± 42.7 eggs in test groups and 916.7 ± 70.4 eggs in the control. However, the volume of the clutches differed significantly and made up 89.5 ± 6.7 and 187.2 ± 14.9 ml, respectively. A stable tendency for a decrease in the dimension of the eggs was noted in the population from the polluted zone. Thus, the average egg diameters at stage 11–12 (Dabagyan and Sleptsova, 1975) amounted to 1.68 ± 0.02 mm (n = 80) in the test group and 1.71 ± 0.01 mm (n = 60) in the control; at stage 16–17, these values were 1.79 ± 0.017 mm (n = 100) and 1.85 ± 0.016 mm (n = 60), respectively. It is necessary to mark the increase in the frequency of layings of undersized embryos in water bodies of the polluted zone. We noted the same phenomenon earlier in populations of *Rana arvalis* Nilss. in urbanized territories (Vershinin and Gatiyatullina, 1994) and in the region of the accident at the Chernobyl Nuclear Power Station (Cherdantsev *et al.*, 1993).

The experiments also revealed that embryo mortality was about 7 times greater in clutches in the polluted territory. Thus, the 30th developmental stage was reached only by 11.82 ± 2.36% of the experimental animals, but this figure was 79.77 ± 5.78% in the control. During the period of egg development, the premature disintegration of egg lumps to single eggs, lavrae falling out of their integuments, and the loss of a significant part of the lavrae were noted. Numerous laboratory observations revealed that 400–800 larvae per female survived on average. The number of larvae from clutches of the polluted region was found to be very low and amounted to 89 specimens per clutch. Thus, investigation of the embryonic development of *Rana arvalis* Nilss. in territory contaminated with radionuclides confirmed our results obtained previously for populations in urbanized and technogenic territories. A common regularity was noted: under the influence of anthropogenic factors, the masses and sizes of eggs diminished, and physiological quality and viability decrease.

After the end of embryogenesis, observations were continued to investigate the survival and development of the emerged larvae. Animals at the 39th stage of development (Dabagyan and Sleptsova, 1975) from six experimental and six control clutches were placed in 3-l vessels (at a density of two larvae per liter) in five replicates for every clutch. Development proceeded at a temperature of 20–22°C, and boiled leaves of dandelion were used as fodder. In the course of the experiment, the development rate, survival during the larval period, and the period of metamorphic climax were determined. Furthermore, 114 specimens that emerged in the current year and 23 adult animals were taken from natural conditions in the test and control lots.

It is known that the support of dynamic polymorphism in amphibian populations is largely determined by the differential vitality of various genotypes at different ontogenetic stages under different environmental conditions (Pikulik, 1978a, 1978b). The ratio of various morphs in populations reflects the specific character of their genetic structures and can be used in ecological monitoring. One of the morphotypes of *Rana arvalis* Nilss., striata (a dorsomedial light-colored stripe on the back), is a result of the action of an autosomic gene upon the total predominance of one of the alleles. The allele determining the existence of the stripe is dominant (Shchupak, 1977). Specimens of this morph have a number of advantageous physiological features under conditions of anthropogenic environments (Gogoleva, 1989).

Several metabolic traits are known that determine the adaptive value of striped specimens under conditions of pollution and urbanization (Dobrinskii and Malafeev, 1974). It was revealed (Usachev *et al.*, 1993) that, during the first year of their life, specimens of the striata morph absorbed ^{90}Sr to a considerably larger extent than specimens without stripes. The authors attributed this fact to distinctions in metabolism. The capacity for the increased accumulation of metals by the striata form was also noted by other researchers (Sharygin, 1980a, 1980b). As a rule, the number of striata specimens is distinctly lower in natural populations with low anthropogenic influence. The high occurrence of the striata morph among individuals that emerged in the current year and adults of *Rana arvalis* Nilss. in the shore region of Lake Berdanish (Table 1) is evidence of purposeful changes in the genetic structure of the investigated population toward the predominance of specimens having a high level of metabolism. However, the occurrence of the striata morphotype among individuals born in the current year developing under laboratory conditions was somewhat different (Table 2). Four out of six control groups gave only stripeless animals; in one test group, their numbers amounted to 65%, and only one test (no. 5) gave 54% striped specimens. Overall, the striata morph amounted to 14.8% in control clutches and 48.2% in experimental groups. Among clutches from Lake Berdanish, one contained no striped specimens, and another contained no stripeless ones. The absence of clearly expressed changes in the ratio of morphs expressed by test groups of animals seems to be related to the selective elimination of different morphotypes under both natural and laboratory conditions.

Table 1. Frequency of the striata morph in *Rana arvalis* Nilss. populations (June), %

Habitat	Animals born in the current year	Adults
Lake Berdanish	53.0 (n = 30)	58.3 (n = 12)
Dolgobrodskoe reservoir	22.0 (n = 54)	18.0 (n = 11)

Table 2. Frequency of the striata morph in animals that emerged in the current year developing under laboratory conditions

Place of egg sampling	Clutch number	Number of animals per test	% striata
Lake Berdanish	1	18	50
	2	22	100
	3	21	52
	4	12	50
	5	24	37
	6	18	0
Dolgobrodskoe reservoir	1–4	71	0
	5	11	54
	6	23	35

We tried to follow the reaction of animals to a change in environmental conditions (the chronological aspect of the problem) by using the method of morpho-physiological indicators (Shvarts *et al.*, 1968). The results of the investigation of some parameters of specimens that emerged in the current year of *Rana arvalis* Nilss. taken from natural conditions (Table 3) indicate a size increase in animals of the population of Lake Berdanish. These distinctions were evidently connected to both the predominant survival of large specimens and specificity of the water bodies where their development took place. The relatively lower liver index ($p \leq 0.01$) probably indicates higher energy expenditures. Such changes reveal the presence of physiological processes manifesting themselves in different metabolic rates in large and small animals. Moreover, it is known that the liver size directly depends on the metabolic rate.

Heart mass is usually determined by some specific traits of animals, including growth rate. It was revealed with mammals that animals having high growth rates differed from others by their large hearts (Shvarts *et al.*, 1968). At the same time, a clear deviation of heart mass from the standard can be connected to disturbances or changes in the environment. Comparison of distribution frequencies of index values for hearts and livers of individuals that emerged in the current year revealed deviations from the standard distribution: specimens with low values for both indices predominated, indicating directional selection pressure. This assumption was confirmed by the absence of such differences among individuals that emerged in the current year under laboratory conditions (Table 4).

Analysis of the morphophisiological indices of adults specimens (Table 5) revealed that animals from the radionuclide-contaminated zone exhibited significantly smaller values than control ones; however, the heart and liver indices manifested negligible differences, if any. The decrease in the relative weight of the spleen can be a feature of depressed hemopoietic activity in animals from the contaminated zone. The small sizes of sires can be connected to a decrease in the lifespan of the reproductive nucleus of the population, which is typically true for polluted territories (Ushakov *et al.*, 1982; Vershinin and Volegova, 1993). The ratio of body mass to length revealed that animal girth on the test ground was essentially lower than in control one and amounted to 123.13 ± 11.8 and 256.6 ± 20.4 arb. units. This can be a result of the predominance of specimens with high levels of metabolism and capable of efficiently removing radionuclides from their organisms or a result of a drop in the abundance of food (insects and other invertebrates) in the radionuclide-polluted territory.

Regression analysis for the dependence of heart, liver, kidney, and spleen size on body length in adult animals established a direct linear relationship between these indices for all cases, but the regression coefficients of animals from the polluted zone and the control were clearly different (Table 5). Values of a and b in the control were close to our data obtained in another pristine region of this landscape. This was a result of both differences in the metabolic level and changes in hemopoietic organs. Distinctions in regression coefficients were also discovered for the relationship between the liver size and body length of animals that emerged in the current year.

The frequency of morphological anomalies can also be used for environmental assessment (Cooke, 1981; Vershinin, 1982). Comparison of the portion anomalies among animals that emerged in the current year revealed that the combined frequency for different types of morphological anomalies amounted to 16.7% ($n = 60$) on the polluted territory and 1.83% ($n = 54$) in the control, which was a result of the unfavorable ecological situation in the Lake Berdanish region. Similar results were obtained for *Rana arvalis* and *R. temporaria* for the Bryanskaya oblast (Kornilova, 1993).

Table 3. Morphological indices ($M \pm m$) of animals that emerged in the current year from natural populations

Place and time of catch	Body mass, mg	Body length, mm	Index, ‰		
			liver	intestine	heart
Lake Berdanish (June 29, 1993)	531.16 ± 27.53	17.86 ± 0.3	53.05 ± 2.0	134.02 ± 4.92	2.97 ± 0.33
Dolgobrodskoe reservoir (June 29, 1993)	306.59 ± 5.59	14.46 ± 0.12	63.49 ± 1.58	133.8 ± 2.18	4.01 ± 0.23

Table 4. Morphophysiological indices ($M \pm m$) of animals that emerged in the current year from the laboratory test

Place and time of egg sampling	Number of animals	Body mass, mg	Body length, mm	Index, ‰	
				liver	intestine
Lake Berdanish (May 6, 1993)	115	178.9 ± 5.46	12.75 ± 0.13	49.83 ± 0.96	111.5 ± 1.26
Dolgobrodskoe reservoir (May 6, 1993)	105	185.99 ± 4.64	12.96 ± 0.11	50.63 ± 0.99	114.1 ± 1.47

Table 5. Data obtained by regression analysis of the ratio of organ mass to body length in adult *Rana arvalis* Nilss.

Organs	Lake Berdanish			Dolgobrodskoe reservoir		
	R	*a*	*b*	*R*	*a*	*b*
Kidneys	0.692	0.96	−19.15	0.876	1.95	−57.40
Spleen	0.41	0.23	−2.63	0.52	2.79	−95.80
Liver	0.877	13.87	−348.83	0.842	31.05	−1091.58
Heart	0.95	1.023	−21.699	0.926	1.88	−52.048

Thus, as a result of a study of the long-term influence of ionizing radiation on *R. arvalis* populations, the predominance of this species in the investigated territory was noted. Changes in the population structure and the appearance of specific phenotypic features predominant in the female portion of the population and a change in the ratio of morphs (an increase in the portion of striata) were discovered. The predominant survival of large individuals that emerged in the current year and small mature specimens compared to the control animals was revealed. This was probably connected with early maturation and a shorter lifespan in the polluted territory. An increase in the frequency of anomalies reflected the potential genetic danger of the environment. Analysis of morphophysiological traits revealed the predominance of animals with high metabolic levels and depressed functioning of their hemopoietic organs. A negative radioactive influence was reflected in lowered gamete fertility, an increase in embryo mortality, a decrease in egg diameter, and a decrease in the general stability of an organism. The complex of characteristics of the population structure formed in the investigated species reveals the predominance of animals differing phenotypically and physiologically from those in natural populations. On the one hand, this is a reflection of the ecological situation under conditions of contanination with radionuclides; on the other hand, this reveals adaptive changes contributing to the existence and reproduction of species under new conditions.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 93-04-7888.

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