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AQUATIC TOXICOLOGY

Accumulation of Radionuclides in the Marsh Frog *Pelophylax ridibundus* in the Zone of a Nuclear Plant[1]

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Abstract—The accumulation of anthropogenic radionuclides ^{90}Sr and ^{134,137}Cs in the marsh frog at various stages of ontogenesis is studied in the Beloyarsk Reservoir (cooling pond of the Beloyarsk nuclear plant). The Verkhnetagilsk Reservoir serves as a control (reference site). The variability of individual morphological indicators and concentrations of radionuclides in adult amphibians of the studied waterbodies is noted. An abnormally high contamination of ^{134}Cs and ^{137}Cs is revealed in one frog from the industrial flood channel of the Beloyarsk Reservoir, which may be due to contact between the animal and a radioactive medium in the area of the nuclear plant. It has been revealed that, currently, at some observation points of the Beloyarsk Reservoir, the levels of ^{90}Sr and ^{137}Cs accumulation in the frogs are significantly higher than in the control reservoir. However, the concentration of radionuclides in frogs is much lower than the permissible level for amphibians.

Keywords: marsh frog, Beloyarsk Reservoir, nuclear power plants, Verkhnetagilsk Reservoir, concentration, ^{90}Sr and ^{137}Cs, aquatic animals

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INTRODUCTION

Interest in the marsh frog (*Pelophylax ridibundus* Pall., 1771) as an object of radioecological research is determined by the wide distribution of this amphibian species in various geographic zones around the globe, their high mobility in water and on land, and their resistance to environmental pollution. The spread of the marsh frog became possible due to human propagation activities and the associated widespread thermal anomalies of anthropogenic origin. The marsh frog appeared in Urals in the 1970s (Vershinin, 2007a). Currently, the frogs are being cultivated in artificial and natural waterbodies in several countries (China, Vietnam, France, Belgium, Portugal, Italy, Spain, Holland, etc.); the harvest of the animals reaches 1000 t per year (Dinh, 2015; Mirzaj, 2003).

Only a few papers deal with radioecological studies in frogs. Data have been published on the accumulation of radionuclides in the emergency zones of nuclear industry enterprises (Beresford et al., 2020; Matsushima et al., 2015; Stark et al., 2004; Stark, 2006; Luneva, 2018). However, there have not been any radioecological studies on frogs in the Urals region, where many nuclear facilities operate (the BNPP, nuclear reactors in scientific and medical institutions, and centers for processing radioactive materials and for the disposal of radioactive wastes); technological nuclear explosions were carried out and a severe radiation disaster occurred at the Mayak facility (Utkin, 2004).

The goal of this paper is to study the levels of accumulation of long-lived radionuclides ^{90}Sr and $^{134,\,137}$Cs in marsh frogs dwelling in the Beloyarsk Reservoir (the BNPP cooling pond) in Urals.

MATERIALS AND METHODS

Marsh frog specimens of different age groups were studied: adults, underyearlings, and larvae. In addition to amphibians, the accumulation of radionuclides is assessed in water, aquatic plants, and bottom sediments sampled in the Beloyarsk Reservoir. The reservoir is located in the Middle Urals, 50 km from Yekaterinburg; it was created in 1959–1963 by damming the Pyshma River at a distance of 75 km from its source. The ecological-geographic and hydrochemical characteristics of the Beloyarsk Reservoir are given in (Trapeznikov et al., 2008). Currently, two power units are operating at the BNPP: the third (since 1980) and the fourth (commissioned in 2014). During the operation of the first three power units, the IFC served as the main route of radionuclide inflow into the Beloyarsk Reservoir. The unbalanced waters (water that had undergone special water treatment, water from special laundries, showers, melt and stormwater from

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[1] **Abbreviations:** BNPP, Beloyarsk nuclear power plant; IFC, industrial flood channel.

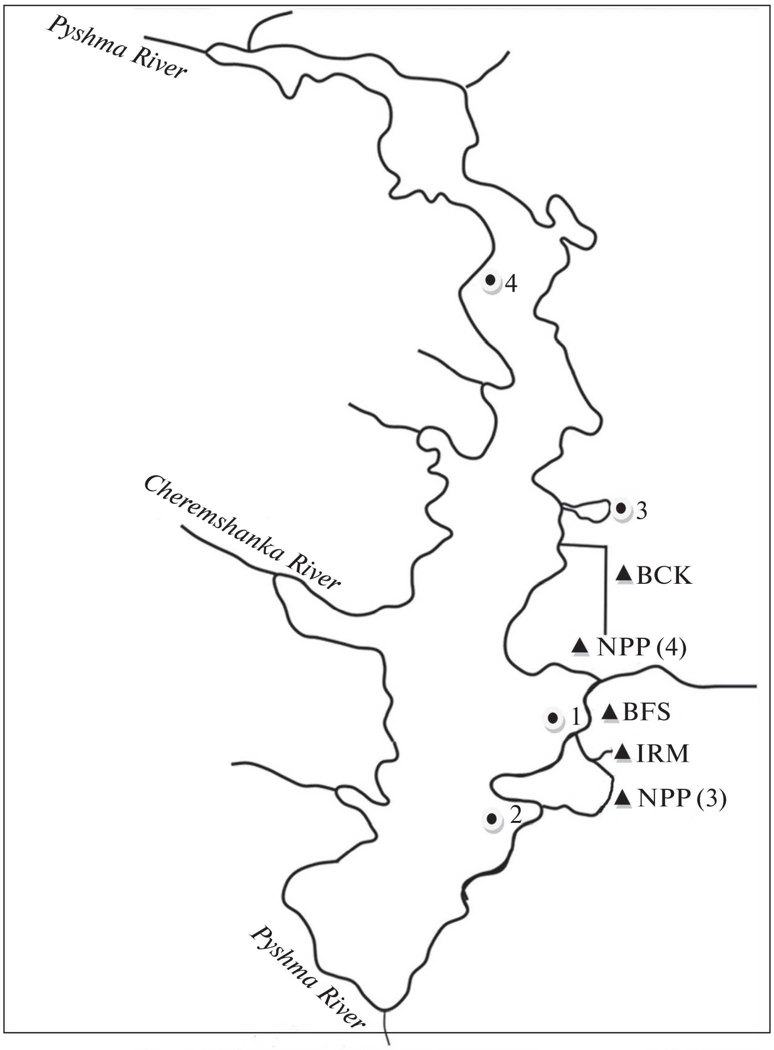

Fig. 1. Schematic map of the Beloyarsk Reservoir. NPP (3) and NPP (4), third and fourth power units of the Beloyarsk nuclear power plant; *3*, water discharge channel from the fourth NPP power unit; IRM, Institute of Reactor Materials; and BFS, biophysical station of the Institute of Ecology of Plants and Animals, Ural Branch, Russian Academy of Sciences. Sampling points: + *1*, industrial flood channel; *2*, zone of heated-water discharge (warm bay); *4*, waterbody behind the fourth power unit; and *5*, region of the Kedrovaya Roshcha recreation center.

the territory of the station) of the power plant were discharged, as were effluents from a neighboring enterprise, the Institute of Reactor materials (IRM), where the experimental reactor operates, through the channel (Fig. 1). IFC is ≤1 m deep and does not freeze in winter. The predominant aquatic plants growing in the
1 channel are fennel-leaved pondweed and cladophora;
1 duckweed, curly-leaved pondweed and Canada water weed are rarer. The plankton consists of 30 phytoplankton and 10 zooplankton species (original data). There are many fish fries in the channel, and crucian carp, bream, chebak, and perch occur. The abundance of marsh frogs, especially during the reproduction period, is noticeable.

The samples in the Beloyarsk Reservoir were collected at four observation points: the IFC (point 1), the zone of heated water discharge (point 2), a small waterbody (connected with the Beloyarsk Reservoir by a shallow channel overgrowing with aquatic plants) (point 3), a forested area behind the fourth power unit (point 4), and in the region of Kedrovaya Roshcha recreation center situated on the right bank and very far from the nuclear power plant (point 5).

For a comparative assessment of the accumulation of radionuclides by the marsh frog, the samples were collected in the Verkhnetagilsk Reservoir, a cooling pond of the Verkhnetagilskaya power plant located in the Sverdlovsk oblast and unaffected by weakly radioactive discharges. The reservoir is a lotic waterbody, created in 1960. It is a shallow with strong water heating. The marsh frog was first discovered in the Verkhnetagilsk Reservoir in 1970. The place where the animals were caught was located near the Verkhnetagilskaya power plant outside the heated-water discharge zone at a distance of 90–100 km from the BNPP.

Various stages of work were carried out from 2015 to 2018. Frogs were sampled using a fishing rod with worms as a bait; frog underyearlings, tadpoles, and fish fries were caught with hand net. The animals were euthanized with ether. In the laboratory, the frogs were weighed and body length; sex; and, in some samples, age were determined. To determine the age, sections of the second phalanx of the fourth toe of the right hind limb of the amphibian were used; the section thickness was 15–18 μm. After decalcification of the fingers in a 5% solution of HNO_3 for 5 h, sections were prepared using a freezing microtome, stained with hematoxylin for 3 min, and placed in glycerol for preservation. In the course of data processing, the outer diameter of the cut and the average diameters of all lines of growth arrest (the lines formed during wintering) were determined. By that means, the age was determined by the number of wintering periods experienced by the animal (Kleinenberg and Smirina, 1969; Smirina, 1972; Castanet and Smirina, 1990).

During the study period, 151 adult frogs were caught in the indicated habitats, including the Verkhnetagilsk Reservoir, of which 45 were females and 106 were males. Four frog underyearlings (total weight 25 g), 95 tadpoles (130 g), and 530 fish fries (375 g) were caught. The age was determined in 11 female and 32 male frogs caught in the IFC: there were 12 1-year-old, 14 2-year-old, 14 3-year-old, 1 4-year-old, and 2 5-year-old frogs.

Plants and fish were sampled in three replicates, 2–3 kg each. Plankton was collected from a layer 0–1 m from the water surface using 67-μm mesh gauze nets. The bottom sediments were collected with a sampler at a depth of 0–5 cm. Water samples (70 L per replicate) were acidified, filtered under laboratory conditions, and evaporated to a dry residue.

After drying and ashing at a temperature of 500°C, the animals were examined for the content of radionuclides. The ^{90}Sr concentration in the ash samples was determined by the radiochemical method (Trapeznikov et al., 2008). Radiometry of the obtained precipitates was carried out on UMF-2000 (Russia) low-background installation in three replicates with a statistical error of 10–15%. The concentration of $^{134,\ 137}$Cs was determined using Canberra Packard and ORTEC (United States) multichannel γ analyzers with a measurement error of 10–20%. During the analysis of samples, ^{134}Cs was found in a significant amount only in one frog from the IFC; in the rest of the samples, the radionuclide was present in microconcentrations below the level of determination confidence. When performing radiometry for ^{90}Sr, each frog was analyzed separately; for $^{134,\ 137}$Cs, three to four samples were combined according to the sex of the frogs to improve the accuracy of determinations.

The results of the study were processed using the correlation and single-factor dispersion analysis.

RESULTS

Table 1 presents the data on the body length and weight of the marsh frog from various observation points. During the study period, frogs from the reservoir behind the fourth power unit had on average a larger body length (by 20%) and body weight (by 47%) than those living in the IFC and the warm bay of the Beloyarsk Reservoir, as well as in the Verkhnetagilsk Reservoir. Presumably, this is determined by the favorable living conditions for frogs in the reservoir located in a forest: the absence of a fast water flow and storm waves, good heating of water at a shallow depth of the reservoir, abundant food supply, etc. In the area of the Kedrovaya Roscha recreation center, we managed to catch only one frog; it had a relatively large body length and weight. Further, due to the lack of a statistically significant group sample, this sampling point was not considered.

As the values of coefficients of variation (C_v) presented in Table 1 show, the content of ^{90}Sr and ^{137}Cs radionuclides in frogs in most habitats is significantly variable. Lower concentrations of ^{137}Cs were noted in frogs from the zone of discharge of heated water from the Beloyarsk Reservoir. These values significantly differ from those of other habitats, including the Verkhnetagilsk Reservoir ($p < 0.0001$).

In addition to those data given in Table 1 in the IFC channel, one frog with a high content of 137Cs (45 000 Bq/kg) and 134Cs (441 Bq/kg) was found.

A comparative analysis of the ^{90}Sr and ^{137}Cs concentrations in the marsh frog and other groups of aquatic organisms in the IFC (Table 2) revealed that both radionuclides accumulate in amphibians, plants, and plankton, on average, at higher levels when compared with fish. Frog underyearlings and tadpoles contain more ^{90}Sr and ^{137}Cs than adults. The components of the IFC ecosystem with increasing concentration of ^{90}Sr and ^{137}Cs are arranged in following order:

^{90}Sr	^{137}Cs
Fish fries	Fish fries
Crucian carp, bream	Bream
Sandy-silt bottom sediments	Crucian carp
Adult frogs	Adult frogs
Tadpoles	Sandy-silt bottom sediments
Fennel-leaved pondweed	Frog underyealrings
Cladophora	Tadpoles
Frog underyealrings	Cladophora and fennel-leaved pondweed
Plankton	Plankton

Table 1. Body length and weight of marsh frogs and body burdens of ^{90}Sr and ^{137}Cs in the Beloyarsk and Verkhnetagilsk reservoirs

Sampling point	Number of specimens	Body length, mm	Body weight, g	Contents of radionuclides, Bq/kg dry weight	
				^{90}Sr	^{137}Cs
Beloyarsk Reservoir					
IFC	43	79.4 ± 1.3 57.0–93.5 (11)	56.6 ± 2.7 21.5–105.5 (34)	9.7 ± 0.9 1.4–24.9 (57)	18.1 ± 1.7 8.0–26.2 (31)
Warm bay	50	75.2 ± 1.2 48.2–86.4 (11)	50.9 ± 1.7 19.1–80.8 (24)	10.2 ± 1.6 1.1–20.6 (75)	4.3 ± 0.65 2.1–7.9 (45)
Bay behind the fourth power unit	21	95.5 ± 1.9 86.0–118.1 (13)	102.2 ± 6.8 75.6–190.1 (33)	6.6 ± 0.4 2.7–9.3 (24)	22.3 ± 2.6 7.3–51.9 (55)
Region of Kedrovaya Roshcha recreation center	1	94.0	116.3	3.2	15.9
Verkhnetagilsk Reservoir					
Region of power plant	36	77.3 ± 1.4 56.0–92.1 (12)	53.7 ± 2.4 19.1–79.9 (29)	7.1 ± 1.2 1.0–20.0 (68)	11.8 ± 1.0 6.5–19.2 (33)

Mean and standard error of mean are on the top; min—max and variation coefficient in parentheses are on the bottom.

No differences in the content of radionuclides in adult frog males and females within the Beloyarsk Reservoir were revealed ($p > 0.05$): 9.8 ± 1.0 and 8.9 ± 2.3 Bq/kg for ^{90}Sr and 17.6 ± 2.4 and 18.7 ± 3.2 Bq/kg for ^{137}Cs, respectively. A statistical processing of the set of data on the reservoir showed a tendency towards a decrease in ^{90}Sr accumulation with an increase in the body weight of the animals ($C_v = -0.314$). Using the IFC as an example, it was found that in adult frogs there is no significant correlation between the body ^{90}Sr and the age of animals from 1 to 4 years ($C_v = 0.043$).

Table 2. Contents of ^{90}Sr and ^{137}Cs in various components of the Beloyarsk Reservoir IFC ecosystem

Study object	^{90}Sr	^{137}Cs
Water	20 ± 3	33 ± 1
Plankton	44 ± 2	2650 ± 306
Tadpoles	15 ± 7	486 ± 61
Frog underyearlings	27 ± 3	397 ± 86
Frogs	9.7 ± 0.9	18 ± 2
Fish fries	0.8 ± 0.5	5.0 ± 2.3
Crucian carp	1.5 ± 0.1	17 ± 1
Bream	1.7 ± 0.7	8.8 ± 1.8
Fennel-leaved pondweed	15 ± 1	1228 ± 179
Cladophora	22 ± 2	1156 ± 150
Sandy-silt bottom sediments	6.4 ± 1.5	81 ± 9

Units of measurement of indicators: water, Bq/m^3; the rest, Bq/kg dry weight.

DISCUSSION

The Beloyarsk Reservoir is an object of comprehensive radioecological research. Several papers (Trapeznikov et al., 2008; Trapeznikov et al., 2015, 2019; Chebotina, Nikolin, 2005; Chebotina et al., 2002) analyzed long-term data on the accumulation levels and dynamics of concentrations of long-lived radionuclides in various components of the cooling reservoir ecosystem (water, plants, plankton, fish, and bottom sediments) in different periods of operation of the nuclear power plant. It was found that, at the first stages of NPP operation, the pollution of the ecosystem of the reservoir with radionuclides was associated with the operation of the first (AMB-100) and second (AMB-200) power units, while the currently operating third (BN-600) and fourth (BN-800) power units did not make a significant contribution to the pollution of the reservoir.

It is known that, under conditions of high radioactive contamination, for example, in accidents at nuclear plants, frogs may accumulate radionuclides in high concentrations. Matsushima et al. (2015) presented data on the accumulation of $^{134,\ 137}$Cs by five frog species in the 20-km zone of the Fukushima NPP and beyond it after the 2011 accident. In this area the content of radionuclides in frogs varied from 3.7 to 47 kBq/kg wet weight. Histological examination revealed no obvious deviations in the morphology of germ cells of frog gonads. Beresford et al. (2020) revealed that, in the exclusion zone of the Chernobyl nuclear power plant in 2014, the concentration of radionuclides in the moor frog were as follows (wet weight): ^{90}Sr from 14.8 to 59.3 kBq/kg, ^{137}Cs from 7.7

to 119 kBq/kg, and ^{241}Am from 0.5 to 9.5 kBq/kg. In the swampy ecosystems of the central–eastern part of Sweden, 17 years after the Chernobyl accident, the average concentration of ^{137}Cs in the moor frog was 1.7 ± 1.1 kBq/kg wet weight, and the highest values (3.5 kBq/kg wet weight) were recorded in the smallest amphibian specimens (Stark et al., 2004; Stark, 2006). The authors estimated the coefficients of radionuclide accumulation, the values of which relative to water were significantly higher than in relation to soil.

O.A. Pyastolova et al. (1996) used amphibians for the purposes of bioindication: changes in the population structure and physiological and genetic differences were revealed in the frogs living in radioactively contaminated areas in the zone of the Mayak PA when compared with the control region. V.L. Vershinin (2007b) revealed alterations in liver, blood, gonads, and life expectancy in the frogs on the territory of Eastern Ural radioactive trace.

To assess the effect of the Beloyarsk NPP on the accumulation of radionuclides by frogs, the data were compared with those in the reference waterbody (the Verkhnetagilsk Reservoir). It was found that the average ^{90}Sr content in frogs of the industrial flood channel and warm bay of the Beloyarsk Reservoir, located 0.5 and 2 km downstream of the BNPP, is ~40% higher than in the control (the difference is highly significant at $p < 0.05$). At a more distant sampling point located 6 km upstream towards the upper reaches (the bay behind the fourth power unit), the ^{90}Sr concentration is close to the level in the reference waterbody ($p > 0.05$). In the frogs of the IFC and the bay behind the fourth power unit, excess concentrations of 53 and 89% of ^{137}Cs relative to the control waterbody were noted, respectively (the difference is significant at $p = 0.002$ and 0.004, respectively).

A case of a high content of ^{137}Cs (45000 Bq/kg) and ^{134}Cs (441 Bq/kg) was recorded in one specimen of a marsh frog sampled in the IFC. This may be the consequence of a close contact between the animal and a radioactive section in the area of the nuclear enterprise (traveling to a radioactively contaminated area, hitting a hot particle, etc.) (Rikhvanov, 1997). According to the currently existing classification, in regards to the cesium content, this frog can be classified as radioactive waste (Resolution ..., 2012).

It is assumed that the frogs dwelling in the Beloyarsk NPP cooling pond accumulate more strontium and cesium than animals from the reference waterbody. According to the accepted standards (100 and 200 Bq/kg for ^{90}Sr and ^{137}Cs, respectively) (Methodical guidelines ..., 1998), the values of the concentrations of these radionuclides in the bulk of the studied frogs of the Beloyarsk Reservoir are significantly lower than the permissible level in food for amphibians.

The low ^{137}Cs concentrations found in the frogs from the heated water discharge zone in the Beloyarsk Reservoir are noteworthy. These values differ significantly differ from those in other habitats, including the Verkhnetagilsk Reservoir ($p < 0.0001$). It should be noted that these data do not agree with the data of laboratory experiments on studying the effect of temperature on the accumulation of radionuclides by various components of the reservoir. It was found that, with an increase in water temperature, in most cases, the level of accumulation of radionuclides by freshwater aquatic organisms and bottom sediments increases, although in some cases temperature conditions do not affect or even reduce accumulation (Kulikov and Chebotina, 1988; Chebotina et al., 2019). At present, we do not have reliable information to explain the phenomenon of decreased ^{137}Cs accumulation in frogs of the heating zone. This may be determined by the specific hydrochemical conditions in the warm bay, which are formed under the influence of heated waste waters entering the bay from the BNPP after passing through the water treatment and reactor cooling systems. This may result in changes in the food base of animals living in the zone of warm-water discharge.

The increased accumulation of ^{90}Sr and ^{137}Cs radionuclides in underyearlings and tadpoles as compared to adult frogs is possibly related to the relatively large contribution of plankton enriched with radionuclides to the diet of juvenile individuals as compared to adult animals. Similar data on a higher ^{137}Cs accumulation by tadpoles when compared to adult animals are found in the Fukushima NPP zone (Tagami et al., 2018). Concentrations of ^{90}Sr and ^{137}Cs in frogs are, on average, higher than in the studied representatives of the fish fauna, which is associated with the species-specific features of aquatic organisms.

The results of the present study revealed that, in all components of the ecosystems of the Beloyarsk and Verkhnetagilsk reservoirs, the content of ^{137}Cs is higher than of ^{90}Sr. One exception is the zone of discharge of heated water in the Beloyarsk Reservoir, where the ^{137}Cs concentration in frogs is approximately 25 times lower than of ^{90}Sr (the difference is significant at $p = 0.01$).

CONCLUSIONS

The marsh frog inhabiting the Beloyarsk Reservoir accumulates long-lived ^{90}Sr and ^{137}Cs radionuclides in high concentrations as compared to the reference (control) reservoir. It has been found that accumulation in adult frogs does not depend on the weight, sex, or age of the animals. Underyearlings and tadpoles contain more ^{90}Sr and ^{137}Cs than adults. The accumulation of ^{137}Cs in adult frogs, underyearlings, and tadpoles is higher than that of ^{90}Sr.

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COMPLIANCE WITH ETHICAL STANDARDS

All relevant international, national, and/or institutional guidelines for the use of animals have been followed.

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SPELL: 1. pondweed