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First record of the Jean Rostand's "anomaly P" in the marsh frog, *Pelophylax ridibundus*, in central Russia

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This is the first record of specific morphological deviations in the marsh frog on the territory of Russia. Similar anomalies were discovered by Jean Rostand in the 1950s in France and named by him as the "anomaly P". Our observations were made on the territory of the Privolzhskaya Lesostep' Nature Reserve (Penza region, Central Russia) in 2016 and 2017. The anomaly P was found in 17.6 % of individuals. Mild forms of the anomaly P (polydactyly and polyphalangy on the hind and fore limbs) were registered in 11.4 % of individuals, whereas severe forms of the anomaly P were found in 6.2 % of all individuals. The potential causes of the anomaly P are discussed.

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INTRODUCTION

Morphological anomalies of amphibians can be caused by various factors, including temperature, pH, illumination, dissolved oxygen and metabolites in water, chemical or radioactive pollution of water bodies, parasites, incorrect regeneration, mutations and others (Dubois 1979; Vershinin 1989; Ouellet *et al*. 1997; Flax & Borkin 1997; Flyaks & Borkin 2004; Nekrasova *et al.* 2007; Lannoo 2008; Borkin *et al.* 2012; Henle *et al.* 2017*a*). However, for most types of anomalies the causes of their occurrence are still unknown (Ouellet 2000; Vershinin 2002; Borkin *et al.* 2012; Henle *et al.* 2017*a*). This includes the "anomaly P", which has an unknown etiology. It was studied in details by the famous French scientist and writer Jean Rostand (1949, 1952). The anomaly P is a bilaterally symmetric mass morphological anomaly that includes special symptoms, such as polydactyly and polyphalangy on anterior and posterior extremities, polymely, tumors and neoplasms in the region of hind limbs, flexion of hind limbs, and others (Rostand 1952, 1959, 1971; Dubois 1979, 2014, 2017). Several specific symptoms distinguish the anomaly P from other types of morphological anomalies. The anomaly P was first found in populations of species of the West Palearctic green frogs of the genus *Pelophylax* Fitzinger, 1843 only and was not revealed in syntopic amphibian species. It is represented by a steep gradient of symptoms from increased number of fingers and toes in mild forms up to flexion of hindlimbs associated with tumor-like structures in femur region, brachymely and polydactyly in severe forms. The severe forms of the anomaly were found in tadpoles and metamorphosed froglets (imagos) only, whereas the mild form was observed in adults as well. Its occurrence often exceeds a level of "background" mutations (conventionally considered to be less than 5 %; Borkin *et al.* 2012). After series of experiments carried out to identify of causes, it was suggested that the anomaly P may be caused by an unknown exogenous teratogenic factor that has effect on limb development (Dubois 1979, 2014, 2017).

The study of the anomaly P was discontinued after Jean Rostand and did not resume for almost 50 years, but identification of its causes has undoubtedly a great interest. The analysis of distributional pattern, types of biotopes and morphological characters in individuals of green frogs with the anomaly P could play an important role for identification of infectious teratogenic agents and developing of measures to prevent of possible epizooties.

In 2016, we found mass morphological anomalies in the marsh frog, *Pelophylax ridibundus* (Pallas, 1771), in Central Russia (Svinin *et al.* 2018). These anomalies were represented by symmetrical polydactyly, polyphalangy, hindlimbs flexions, outgrows and specific neoplasms. Such symptoms are typical for the anomaly P. In this paper, we describe the first results of our two-year study and discuss about potential causes of the anomaly.

Material and methods

Our study was conducted from May to October in 2016 and 2017. In total, ten water objects (water bodies and streams) were examined in the valley of the Khoper River (52°48'58.4"N, 44°27'40.4"E) in the "Ostrovtsovskaya Lesostep'" part of the "Privolzhskaya Lesostep" Nature Reserve (Penza region, Russia). The locality is situated in the upper part of the Khoper River and represented by forest-steppe landscapes, which includes the valley of two small rivers Selimutka and Yuzhnaya surrounded by agricultural fields (Fig. 1). This is a system of oxbows consisting of "forest" and "open" parts. The forest part includes permanent water bodies in the center of mixed forest and open oxbows on edge of the forest. This part is surrounded by meadows, shrubs and fields. In some years, some local water bodies may be dry (Bashinskiy *et al.* 2018). Beavers inhabit local small rivers and, therefore, most of their parts are impounded (3.7–6.4 dams per km, according to Bashinskiy & Osipov 2018). The area of these ponds varies from 400 to about 8000 m^2 with depths up to 2 m. Several dams and old artificial reservoirs are in the upper parts of these rivers as well.

In total, 226 individuals of the marsh frog of different age groups (tadpoles, imagos, juveniles, and adults) were collected using visual and dip-net surveys (Heyer *et al*. 1994). In the field, a preliminary identification of species was made using morphological features. Later, species identification of some individuals was performed in a lab by flow DNA cytometry (Borkin *et al.* 1987; Vinogradov *et al.* 1990, 1991) and PCR methods (Ermakov *et al.* 2013). For the last method, phalanges of hind limbs of green frogs were fixed in 96 % ethanol and then were used as tissue samples for DNA analyses. The fragment of the first subunit of the cytochrome c-oxidase (COI) and the first intron of the serum albumin (SAI-1) genes were used as mitochondrial and nuclear (respectively) markers for green frog species identification. The registration and classification of anomalies were held using standard methods (Nekrasova 2008; Henle *et al.* 2017; 2017*a*). Each individual with anomaly P was anaesthetized by submersion in a 1 % solution of 3-aminobenzoic acid ethyl ester (MS 222). After anesthesia specimens were preserved in 70 % ethanol.

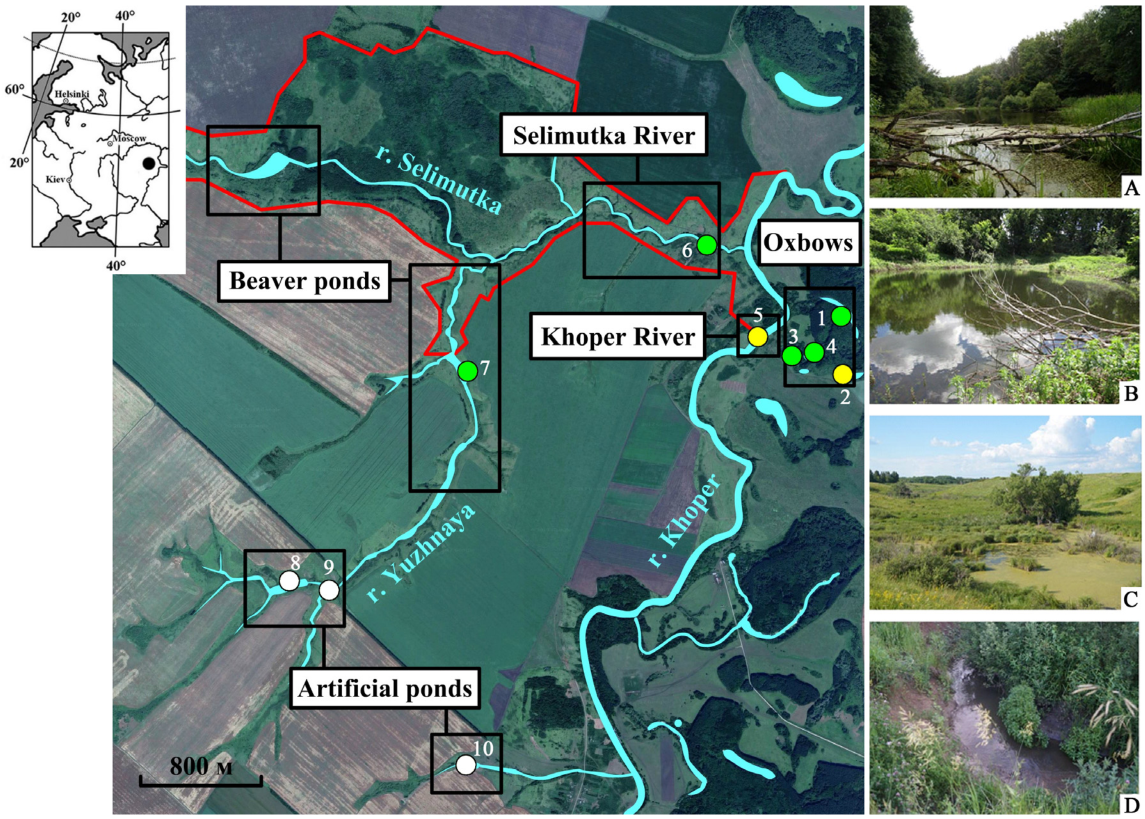

Figure 1. The map of vicinities of the "Ostrovtsovskaya Lesostep'" part of the "Privolzhskaya Lesostep" Nature Reserve with studied sites. Yellow dots are localities, where the anomaly P was found. Red line is a border of the nature reserve. Pictures of studied biotopes: A is a forest oxbow; B is an "open" forest oxbow; C is a beaver pond; and D is the Selimutka River.

Water bodies were studied for measure of abiotic parameters monthly from April to October 2016 and 2017. The following characteristics were surveyed: size and depth of water bodies, temperature of water, amount of dissolved oxygen (Hanna Instruments Dissolved Oxygen Meter HI-9142), pH (Hanna Instruments Portable pH/ORP/EC/Temp "Water Test" Meter HI 98204) and lighting (luxmeter Testo 540). Concentration of biogenic elements (nitrites, nitrates, ammonium, phosphates and chlorides) in water was measured with use of the photocolorimeter "Ecotest-2020" in May and August 2016 and 2017. We tested water from four water bodies to study concentration of heavy metals using of the X-ray fluorescence spectroscopy and the optical emission spectrometry. Additionally, the same analyses were held for ten samples of marsh frog's livers. Heavy metal concentration factors (HMC) was calculated as the ratio between average metal content in an organ to heavy metal content in waterbodies (Flyaks & Borkin 2004).

Statistical analyses were made by use of standard procedures (Sokal & Rohlf 1981). We used Chi-square test with the Yates correction for comparison of frequencies.

Results

A reliable determination of green frog species studied by flow DNA cytometry (n = 4) and PCR (n = 25) analyses revealed that only the marsh frog inhabited the "Ostrovtsovskaya Lesostep" part of the "Privolzhskaya Lesostep" Nature Reserve. Thus, we can state that this territory is inhabited by a "pure" population of the marsh frog. According to the SAI-1 alleles analysis, all specimens with the anomaly P (n = 15) belong to the "western" form of *P. ridibundus* (Ermakov *et al.* 2013). However, the analysis of mitochondrial COI marker showed that 78 % of these abnormal individuals had haplotypes of the "eastern" ("*P.* cf. *bedriagae*") and only 22 % of the "western" forms.

Anomalies were found in 19.5 % of studied individuals of *P. ridibundus* (Table 1). The presence of anomalies was revealed in almost all age groups. However, their number was maximal in tadpoles (larvae) and imagos if compared with juvenile and adult specimens (Yates corrected χ^2 = 5.36; p = 0.021). The severe forms of the anomaly P were found in tadpoles and imagos only, whereas juvenile and adult individuals showed polydactyly, polyphalangy and brachydactyly in one case only. In each of these age groups, the occurrence of anomalies exceeded the conventional threshold in 5 % (in exception of adults in 2016 and juveniles in 2017).

We caught 26 individuals (11.5 %) of frogs with presumed *mild form* of the anomaly P (polydactyly). The individuals with polydactyly were found in five studied water objects. Frogs with anomalies were revealed in standing water bodies (three oxbows of the Khoper River, and beaver ponds) and neighboring Selimutka River (Fig. 1). All such abnormal individuals had polydactyly on hind limbs (11.5 % of studied individuals and 54.6 % of scored anomalies), five of them had also polyphalangy on hind limbs (2.2 % and 11.4 %, respectively), three individuals had polyphalangy on forelimbs (1.3 % and 6.8 %, respectively) and one had brachydactyly on hind limbs (0.4 % and 2.2 %, respectively). All cases of polydactyly (syndactyly, polyphalangy) and brachydactyly were bilaterally symmetric. The number of fingers in these individuals was 6–7 on hind limbs and 5 on forelimbs. The specimens that have polyphalangy on forelimbs always had polydactyly and polyphalangy on hind limbs.

Three imagos (6.8 %; Table 2, no. 6) had abnormal pigmentation of skin between forelimbs. Abnormal pigmentation looked like two crossed thin skin folds between the forelimbs and we called this anomaly "cross" (see fig. 2B).

During our study, we registered 14 *severe cases* of the anomaly P in the "Ostrovtsovskaya Lesostep'" (Fig. 1; Table 3). We found a separate detached right hind limb (counted by us as one individual). All individuals with the severe forms of anomaly P were caught in four biotopes: (1) a forest oxbow (6 individuals); (2) an open oxbow (7 individuals) and (3) an adjacent open oxbow (an individual); and (4) beaver ponds (an individual).

Individuals with the severe forms of the anomaly P had the following characteristics: shortened sections of hind limbs (brachymely), flexions and "outgrows" of hind limbs, neoplasms, an increased number of toes and fingers (polydactyly) and phalanges of toes (polyphalangy) both in the fore (from 5 to 6) and hind (from 6 to 15) limbs (Table 3, Fig. 2). Various neoplasms were observed in some cases, sometimes representing additional distal fragments of limbs ("paws") closely adjacent to inguinal regions of limbs (Fig. 2D). Sometimes, several of these symptoms were lacking in tadpoles in early stages of metamorphosis (Table 3 and Fig. 2). The movements were uneasy in imagos with the anomaly P.

Individuals of other syntopic amphibian species had no such anomalies. We studied 48 specimens of the moor frog, *Rana arvalis* Nilsson, 1842, which were without polydactyly. No limb anomalies were observed in 22 studied individuals of the fire-bellied toad, *Bombina bombina* (Linnaeus, 1761). In this species, a single specimen was missing its left forelimb (amely). However, some tadpoles of the Pallas's spadefoot toad, *Pelobates vespertinus* (Pallas, 1771), had red "swellings" in eyes and abnormal behavior (Svinin *et al.* 2018).

Investigation on chemical pollution of waterbodies

In May and August 2016 and 2017, we measured the concentration of biogenic elements in all types of frog habitats. In 2016, the concentration of phosphates strongly exceeded the standard water quality for fish-breeding ponds in Russia (from 6.17–7.89 to 10.11–16.74 mg/l, conventional threshold limit value (TLV) is 3.5 mg/l; Anonymous 2016). As a rule, this increase is associated with anthropogenic impact, including fertilization of fields. Additionally, high concentration of nitrites (up to 3.56–5.14 mg/l with TLV 3.3) and ammonia (up to 2.39–8.29 mg/l with TLV 2) was observed. High concentrations of NO_2^- and small of NO_3^- ions show that a lot of organic matter did not reach final mineralization. In 2017, we did not observe increased concentrations of biogenic elements during summer, but concentrations of NO_2^-, NH_4^+, PO_4^- and Cl^- exceeded the upper conventional threshold (Table 4).

The concentrations of various elements, including heavy metals, found in water samples from studied habitats with severe forms of anomaly P are shown in Table 4. For 7 of 21 elements, the threshold limit values from the water quality standards were exceeded. Additionally, excess values were observed for P, Cl, Mn, Fe, Cu, Zn and Pb. The increased concentration of chlorine was also observed in biogenic element analysis.

Table 1. The frequency of *Pelophylax ridibundus* with anomalies in the "Privolzhskaya Lesostep" Nature Reserve in 2016 and 2017.

Age groups	2016			2017			Both years		
		With anomalies			With anomalies			With anomalies	
	n	*n*	%	*n*	*n*	%	*n*	*n*	%
Larva	7	3	42.9	12	11	91.7	19	14	73.7
Imago	76	13	17.1	27	6	22.2	103	19	18.4
Juvenile	14	5	35.7	46	1	2.2	60	6	10.0
Adult	13	–	–	31	5	16.1	44	5	11.4
Total	110	21	19.1	116	23	19.8	226	44	19.5

Table 2. The frequency of different morphological anomalies of the marsh frog's individuals.

Type of an anomaly	Absolute and relative (% of total number) occurrence				
	larvae	imagos	juveniles	adults	Total
Mild forms of anomaly P					
Polydactyly of hind limbs	–	12	5	5	22 (9.7)
Polydactyly of hind limbs and forelimbs	–	2	1	–	3 (1.3)
Polydactyly (syndactyly) of hind limbs and forelimbs and polymely (fig. 2*C*)	–	1	–	–	1 (0.4)
Total		15	6	5	26 (11.4)
Severe forms of anomaly P					
Total	14	–	–	–	14 (6.2)
Total mild (polydactyly) and severe forms of the anomaly P	14	15	6	5	40 (17.6)
Other types of anomalies					
Mandibular hypoplasy	–	1	–	–	1 (0.4)
Abnormal pigmentation of skin between forelimbs (anomaly "cross")	–	3	–	–	3 (1.3)
Total	–	4	–	–	4 (1.8)
Total	14 (6.2)	19 (8.4)	6 (2.7)	5 (2.2)	44 (19.5)

The concentration of heavy metals in the liver of studied individuals of the marsh frog, which were caught in "open" forest oxbow, varied from 53 mg/kg (for Cr) to 4215 mg/kg (for Fe). To assess a degree of accumulation, heavy metal concentration factors were calculated (Table 5; Flyaks & Borkin 2004). In our study, the accumulation level was lower for zinc (620) and the highest for copper (14591).

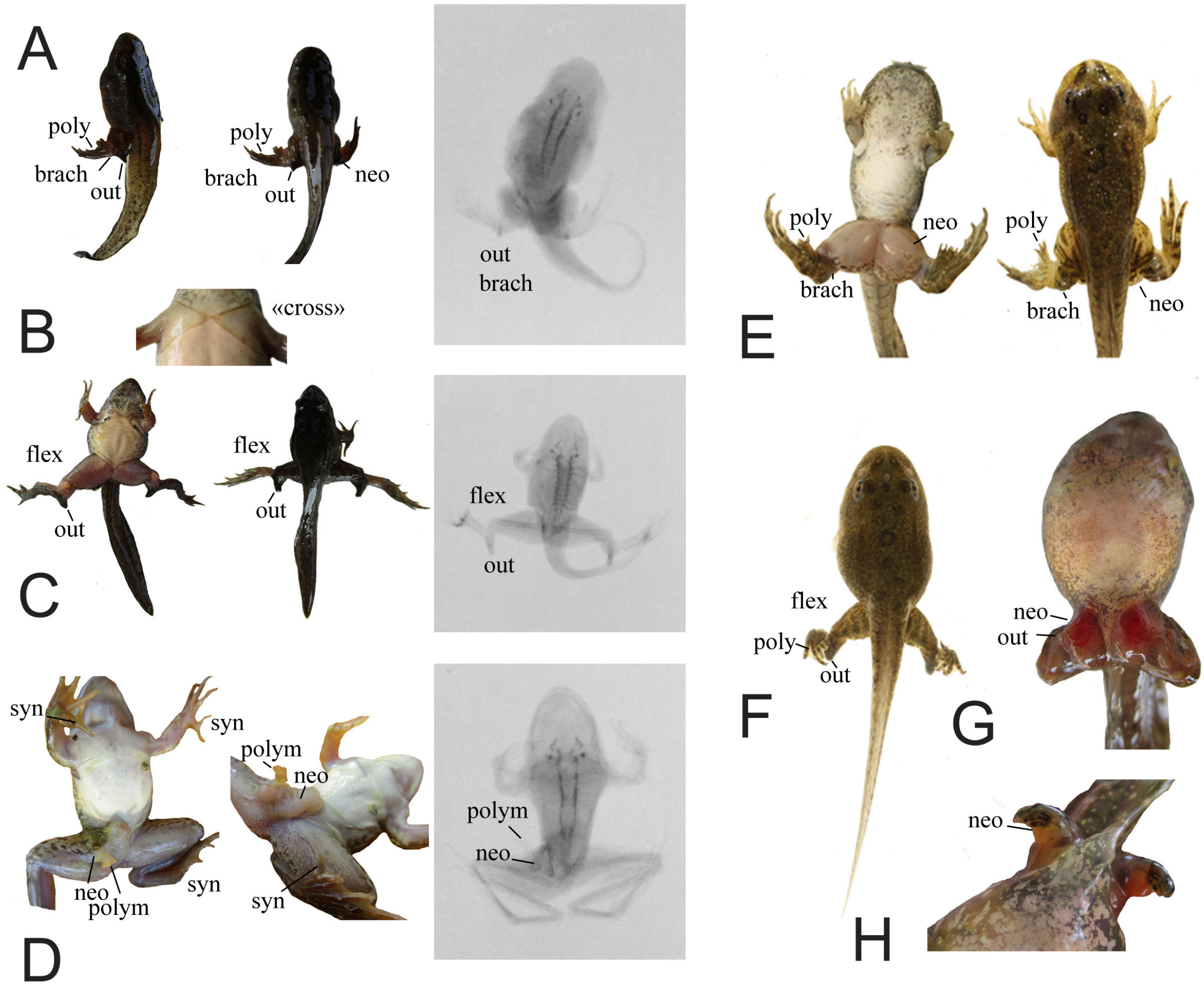

Figure 2. Photographs and radiographs of some individuals of the marsh frog with the anomaly P from the "Privolzhskaya Lesostep" Nature Reserve: A, specimen with flexion, outgrows and polydactyly of hind limbs; B, individual with abnormal pigmentation of skin (anomaly "cross"); C, specimen with normal number of fingers and toes, but with flexion and outgrows of hind limbs; D, new type of the anomaly P from beaver pond in juvenile with symmetrical syndactyly on hind and fore limbs, neoplasms with new developed hind limb in inguinal region; E, individual with polydactyly and polyphalangy on hind and fore limbs, flexion of hind limbs; F, G, H, the anomaly P in early stages: flexions, outgrows and polydactyly of hind limbs (F); tadpoles with deformed hind limbs (G) and neoplasms (H); brach, brachymely; flex, flexions of hind limbs; neo, neoplasm, out, outgrows; poly, polydactyly; polym, polymely; syn, syndactyly.

Table 3. Morphological characters of individuals with severe forms of anomaly P.

$N_{ind.}$	Stage[1]	Polydactyly (number of toes and fingers)				Syndactyly of fingers	Brachymely	Flexion and “outgrows” of hind limbs
		Hindlimbs		Forelimbs				
		Left	Right	Left	Right			
1	40	12	12	–	–	–	+	+
2	40	15	14	–	–	–	+	+
3	?	–	8[2]	–	–	–	+	+
4	42	15	15	6	6	+	+	–
5	42	9	10	6	6	+	+	–
6	44	7	7	normal	normal	–	+	+
7	42	9	10	5	5	+	+	+
8	42	6	6	normal	normal	–	+	+
9	45	normal	normal	normal	normal	–	+	+
10	40	11	11	–	–	–	+	–
11	42	8	8	5	normal	+	+	+
12	40	6	6	–	–	–	+	+
13	45	normal	normal	normal	normal	–	+	+
14	45	6	8	5 (+1)[3]	5	–	+	–

[1] Developmental stage by Gosner 1960; [2] we found only one detached leg with morphological traits of severe form of anomaly P; [3] one additional finger on the left arm.

DISCUSSION

Morphological anomalies in natural populations of amphibians are most frequent if compared with other groups of vertebrates (Dubois 1979; Vershinin 1989). Normally, the upper threshold of occurrence of anomalies in amphibian natural populations is less than 5 % (Borkin *et al.* 2012). The same level was observed as a norm in laboratory experiments (Kovalenko 2000, 2001). However, sometimes the threshold can be exceeded in some natural populations where mass anomalies are observed. The study of the causes of mass anomalies could help to estimate the possibility to use them for bioindication (Pyastolova *et al.* 1996; Vershinin 2014; Borkin 2014; Henle *et al.* 2017*a*).

Mass anomalies in the West Palearctic green frogs are more common than in other European amphibian groups (Bezman-Moseiko *et al.* 2014; Borkin 2014). The first known cases of morphological anomalies in green frogs were found in single specimens. For example, N.A. Kholodkovsky (1896) described polydactyly in two green frog specimens from “Khrenovskiy Bor” forest in Voronezh region (Russia). One of the first cases of mass anomalies in green frogs was found in 1947 by A. A. Voitkevich (1948). He discovered mass polymely in introduced populations of the marsh frog in the Zailiyskiy Alatau Mountains in Kazakhstan. This case was comprehensively studied for a long time, but the causes of these anomalies were not found (Voitkevich 1948, 1958, 1965; Woitkewitch 1961).

Table 4. The concentration of biogenic elements and various chemical elements (mg/l) in water of four water bodies from the "Ostrovtsovskaya Lesostep" with conventional water quality standards for fish-breeding ponds in Russia (Anonymous 2016).

Elements	Threshold limit value	"Open" oxbow	"Forest" oxbow	Beaver pond
Biogenic elements** (May-August 2016-2017)				
NO^{3-}	45	0.86 – **47***	1.06 – 23.18	0.86 – 22.14
NO^{2-}	3.3	0.84 – **3.65**	0.15 – **5.22**	1.06 – **7.18**
NH^{4+}	2.0	1.12 – **8.29**	**2.17 – 7.16**	0.11 – **7.15**
PO_4^{3-}	3.5	1.97 – **26.14**	1.08 – **12.82**	2.15 – **25.15**
Cl^-	350	0.09 – **400.13**	0.14 – **720.13**	0.18 – **699.13**
Various chemical elements (July 2017)				
Mg	40	0.652	0.473	2.020
Al	0.04	0.020	0.022	0.321
Si	–	0.00015	0.00018	0.00074
P	0.00001	**0.040**	**0.036**	**0.099**
S	10	0.017	0.016	2.290
Cl	0.00001	**0.496**	**0.558**	**1.390**
K	50	1.030	1.120	0.951
Ca	180	29.4	35.7	101.6
Ti	0.06	–	–	0.0027
Cr	0.07	0.011	0.019	0.022
Mn	0.01	**0.226**	**0.127**	**3.060**
Fe	0.1	**5.19**	**3.42**	**40.80**
Co	0.01	–	–	–
Ni	0.01	–	–	0.00029
Cu	0.001	**0.137**	**0.052**	**0.022**
Zn	0.01	**0.405**	**0.399**	**0.760**
As	0.01	–	–	0.000038
Sr	0.4	0.138	0.136	0.211
Cd	0.005	–	–	0.000108
Ba	0.74	0.045	0.058	0.059
Pb	0.006	**0.139**	**0.008**	**0.011**

*Extreme values of biogenic elements;
**The values greater than the conventional threshold limit value are shown in bold.

Table 5. Concentration of heavy metals (mg/kg) in liver and heavy metal concentration factors (HMC) of *Pelophylax ridibundus* specimens from "open" forest oxbows.

Elements	mean ± SE	Lim	HMC	HMC (Flyaks & Borkin 2004)
Cu	1999 ± 112	1815 – 2135	**14591**	24 – 1788
Zn	251 ± 28	219 – 289	620	13 – 2684
Fe	4215 ± 182	3986 – 4494	812	213 – 17671
Cr	53 ± 10	38 – 65	4818	–
Mn	230 ± 51	139 – 301	**1018**	16 – 426

* The values greater than HMC from Flyaks & Borkin 2004 shown in bold.

In 1948, J. Rostand (1949, 1952) found mass polydactyly in "*Rana esculenta*" from the vicinities of Trévignon ("étangs de Trévignon et étangs de Penloc'h", Brittany, France). In 1952, he found that polydactyly in these populations was just a 'mild' symptom of a complex syndrome of anomalies which he called "the anomaly P" (Rostand 1952). This polymorphic syndrome involves a wide range of anomalies concerning the limbs (posterior and anterior polydactyly, brachymely, polymely) and the inguinal region (bony excrescences and neoplasms). The anomaly P, or its mild form polydactyly, were reported from 12 localities from France (Bonnet & Rey 1937; Rostand 1962; Dubois 1984) and from other countries (complete syndrome: Austria, Belarus, Germany, Switzerland, Turkey; simple polydactyly in adults: Greece, the Netherlands, Poland and Russia) (Dubois 1984, 2017).

In eastern Europe, cases of polydactyly (mass occurrences and single cases) were recorded in many parts of the distributional range of green frogs (Lada 1999; Zamaletdinov 2003, 2014; Flyaks & Borkin 2004; Kurtjak 2005; Zaks 2008; Spirina 2009; Fayzulin 2012; Korzikov & Alekseev 2014; Bezman-Moseiko *et al.* 2014; Kozhevnikova & Lada 2016). It is still unknown whether all these cases should be attributed to the anomaly P. However, no mass occurrences of anomaly P in tadpoles and imagos were recorded.

During the research on the causes of the anomaly P, a large number of hypotheses were proposed (Dubois 2017). In a series of experiments, J. Rostand (1950*a*) showed that the anomaly P is not inherited and, therefore, the causing factor is environmental. He considered that one of the possible causes for occurrence of the anomaly could be chemical pollution.

The results we obtained in the populations we studied on biogenic elements and heavy metals content exceed the conventional threshold value and show the presence of the anthropogenic factors in the studied biotopes. In a number of other nature reserves, similar concentrations of heavy metals were observed (Flyaks & Borkin 2004; Jayawardena *et al.* 2017; Prokić *et al.* 2017; Zhelev *et al.* 2017). In some previously studied localities, the level of heavy metals pollution was much higher than in our research, but the anomaly P was not found. For example, according to N. L. Flyaks & L. J. Borkin (1997, 2004), concentrations of various heavy metals exceed our values in

Nikopol and Dnepropetrovsk cities (Ukraine), but the anomaly P was not observed in these localities despite the purposeful search for anomalies.

Moreover, in experiments by J. Rostand, none of the chemicals and physical agents that were tested on tadpoles resulted in any of anomalies identical to those of the anomaly P (Rostand, 1950*a*, *b*). This led Rostand (1952) to hypothesize a biotic cause for the anomaly P, and more specifically an “infectious agent” which might be a virus (Rostand 1959; Dubois 1979, 2017).

Some researchers also tended to explain some mass anomalies (including polydactyly, polyphalangy and ectromely) by viral origin (Bezman-Moseyko *et al.* 2014). A number of other studies have revealed that some forms of cancer in amphibians are caused by viruses. For example, the Lucke virus leads to a kidney tumor and chondrosarcoma (McKinnell 1973; Mizgireuv *et al.* 1984) in the northern leopard frog, *Lithobates pipiens* (Schreber, 1782). After experiments in which tadpoles were raised with eels, *Tinca tinca* (Linnaeus, 1758), and tenches, *Anguilla anguilla* (Linnaeus, 1758), or with fish excrements and intestine contents (Rostand & Darré 1967, 1968, 1969), it was assumed that fish, or some components of their diet, might be vectors of a teratogenic virus causing the anomaly P – which does not mean however that other vectors could not play this role. But these results could not be repeated in other experiments (Dubois 1979, 2017).

In our field study we obtained some data that do not support the fish hypothesis. Most anomalies were observed in an “open” oxbow, where for a long time fishes were not detected. In 2016–2017, additional ichthyological studies were carried out and this investigation confirmed this absence of fishes in this open oxbow (Bashinskiy *et al.* 2018). This oxbow was not connected with oxbows of the Khoper River (where fishes are present) even during spring floods.

The distance between the localities where anomaly P was recorded so far is very large. It is most likely that the anomaly is much more widely distributed in Europe than is currently known. This may be a more widespread phenomenon, which might have different manifestations, possibly under the influence of anthropogenic factors. Among the many questions that remain open regarding this anomaly, the reason why only green frogs of the genus *Pelophylax* are affected in a crucial one (Dubois 2017). There remains indeed a lot of work to do to know more about this intriguing syndrome.

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