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A GIS approach to the study of colour anomalies in amphibians of Ukraine reveals the deleterious effect of human impacts

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Abstract. Our study provides a review of colour anomalies in amphibians from Ukraine during the 20th and early 21st centuries. Observations including melanism, flavinism, leucism, and blue axanthism were assembled from the published literature (1909–2018) and during field surveys (2000–2017). Blue colouration was the most common abnormal variant (81.5%; n = 106), and colour anomalies were recorded in 13 of Ukraine's 24 administrative regions (oblasts), mainly along the Dnieper River and in the Carpathian Mountains. The largest number of anomalies was found in the Poltava (26.5%) and Kyiv (20.4%) Oblasts. We also explored the relationship between abnormal colouration and environmental variables using a GIS framework. Correlations existed mainly with geographic location and temperature-related parameters (e.g., reference evapotranspiration), as could be expected for interactions of morphological or physiological anomalies. However, the Human Footprint, an integrated index of anthropogenic impact, was also important. The connection of colour anomalies and human activities shows once again the importance of amphibians as bioindicators for the early detection of pollution and other harmful effects in aquatic ecosystems.

Keywords. Abnormal colouration, blue frogs, blue axanthism, leucism, albinism, Ranidae, Salamandridae, Bombinatoridae, Bufonidae, Human Footprint, pollution, bioindication

Introduction

Body colour plays a significant role in the lives of amphibians, whose interaction with solar radiation has important implications for their thermal biology and correlated survival rate (e.g., Terentiev, 1950; Childs, 1953; Vences et al., 2002; Sanabria et al., 2014; Stuart-Fox et al., 2017; Martínez-Freiría et al., 2020; Smith et al., 2021). Thus, studying amphibian body colour is useful to gain a more in-depth understanding of the phenotypic plasticity and ecological adaptability of these organisms (e.g., Boero, 2013; Kolenda et al., 2017). In recent decades morphological abnormalities, including those involving body colour, have become a common issue for almost all amphibian populations in Ukraine, apparently as a consequence of habitat degradation (Henle et al., 2017a; Reshetylo et al., 2019; Palamarenko, 2020), and it is important to document these and recognise their origins.

Amphibians are known to be affected directly and indirectly by exposure to pollutants or land-use changes, which can result in internal and external morphological abnormalities (Marushchak et al., 2017; Henle et al., 2017a; Marushchak and Muravynets, 2018; Hegde et al., 2019). Of these, colour anomalies are just one group of skin morphology aberrations that were classified as Type S1 ("skin malformations") by Nekrasova (2008). However, determining the origin of a particular anomaly

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is difficult, as these can derive from the combined effects of several factors (Vershinin, 2015; Henle et al., 2017a, c; Miura, 2018). Even though a direct statistical connection between manifestation of colour anomalies and anthropogenic impact has remained elusive (but see Henle et al., 2017c), colour anomalies are being considered signals of environmental distress (Dubois and Ohler, 2018).

Pigment cells. The colour of amphibians depends on the type and distribution of pigment cells in the dermis of their skin, as well as on their ability to manipulate the expansion and distribution of the specific pigments produced (Frost-Mason et al., 1994; Nilsson Sköld et al., 2012). The most important pigment cells are (1) xanthophores, with a yellow or reddish pigment (xanthine); (2) iridophores, which contain plates of crystalline chromatophores that may produce blue colouration (iridescence) due to the scattering of blue wavelengths from their surface (Bagnara et al., 2007); and (3) melanophores, containing melanin, the brown or black pigment (Terentiev, 1950; Britton, 1986). The colouration of an individual depends on the quantity of chromatophores and the pigment distribution inside them (Frost-Mason et al., 1994; Nilsson Sköld et al., 2012).

Colour plasticity. Some amphibian species have gained an adaptation that allows them to change their colour depending on climate conditions, hormonal state, or the substrate on which they sit. For example, males of *Rana arvalis* can display a completely blue body colour during the mating season (a phenomenon termed dynamic sexual dichromatism; e.g., Sztatecsny et al. 2012; Bell et al., 2017), while *Hyla orientalis* can change colour depending on temperature, humidity, and surrounding foliage (Terentiev, 1950; Henle et al., 2017b). Sometimes such changes have been mistakenly interpreted as abnormal cases (Kolenda et al., 2017), but these are excluded in this study.

Colour anomalies. Abnormal colouration will occur when pigment synthesis is disrupted or modified, or when chromatophores are absent, reduced in number, or malfunction (Frost-Mason et al., 1994). For example, the malfunction of melanophores can manifest itself as a paler colour (with a reduction of melanophore activity) or a white, yellowish, or pinkish colour (signalling the complete lack of melanophore activity), which may appear as either monotypic (with only one part of the body or the entire body coloured abnormally, as in albinism or leucism) or polytypic (with multiple areas of the body coloured abnormally, as in so-called mosaic albinism; Nekrasova, 2014). Any genetic or environmental disturbance in the synthesis of pigments or the inactivation or destruction of chromatophores can lead to the appearance of abnormal colouration (Frost-Mason et al., 1994).

Following the unified terminology proposed by Henle et al. (2017b), there are 42 main categories and subcategories of colour anomalies, from the lack of colour in albinism to an extreme concentration of dark colour in hyperpigmentation, that may occur in amphibians. Albinism and leucism – a subcategory of albinism, in which individuals have a white body but normal coloured eyes – are the most commonly described colour anomalies in the literature for both anurans and urodeles (Henle et al., 2017a), but they are only rarely reported from Eastern Europe and European part of Russia. Albinism is known for the anurans *Bufo bufo* (Wenig, 1913; Kaczmarski, 2018), *Pelophylax lessonae* (Dandová et al., 1995; Necas et al., 1997; Lada et al., 2008; Kuzmin, 2013), *P. ridibundus* (Borkin et al. 1981, 1982; Lusis & Tsaune 1984; Gabriel, 1987; Mikulíček et al., 2001; Pabijan et al., 2004; Adlassnig et al., 2013), *Rana temporaria* (Sosnovskii, 1958), and *Rana arvalis* (Kaczmarski and Kaczmarek, 2018) and for the urodeles *Salamandra salamandra* (Vlad et al., 2020), *Lissotriton montandoni* (Skorinov, 2009), *L. vulgaris* (Necas et al., 1996; Kopecký et al., 2013), and *Triturus karelinii* (Göhler, 1981). Abnormal blue colour is much more rarely reported for amphibians, primarily for frogs in the genera *Hyla* and *Pelophylax* (Henle et al., 2017a). Despite the fact that blue frogs are considered to be a special type of axanthism, this term is seldom applied to such abnormally coloured amphibians.

Other than the expected, stochastic occurrence of mutations, mutagenic chemicals and radioactivity can cause anomalies on a genetic level and physiologically (e.g., Hayes and Hansen, 2017; Henle et al., 2017c; Gombeau et al., 2020). While mutagenic activity by agricultural chemicals remains controversial, some combinations (e.g., carbaryl in combination with atrazine) definitely have this potential (Bridges et al., 2004). Iris depigmentation was reported for *Rana arvalis* in areas polluted by radioactivity, and although this would presumably be manifested via genetic pathways, its prevalence appears to have been modulated, perhaps via mortality and/or by low temperatures, including cold spells in springtime (Vershinin, 2004, 2006). We do not know as much about non-genetic causes than about genetic ones for colour anomalies. However, some agrochemical and industrial pollutants (carbamate, dichlorvos, lindane, glyphosate) are known to cause primarily darkening and bleaching of colouration (Marchal-Ségault and Ramade, 1981; Tomar and Pandey, 1985; Raj et al., 1988; Henle et al., 2017c; Smith et al., 2021).

Our study aims to summarise the available data regarding the occurrence of abnormal colour patterns recorded in amphibians of Ukraine and analyse the frequency of occurrence of these anomalies using a GIS approach so that it might be possible to find environmental correlates.

Materials and Methods

Field sampling. Records of colour anomalies in amphibians were obtained opportunistically during fieldwork from 2000–2017 in 25 administrative units (known as oblasts) of Ukraine. A total of 9892 frogs and salamanders were examined for signs of abnormal colouration. Amphibians were caught by hand with the help of dip nets in their typical habitats from March–October of all years during which fieldwork was conducted. Individuals with colour anomalies were photographed. Colour anomalies were determined following Henle et al. (2017b). Blue-coloured individuals were captured and monitored for one month in the laboratory (n = 74), or those that could not be captured were monitored for two weeks in their natural habitat by visually surveying for blue specimens in the water body where they were initially found (n = 32), to determine whether the blue colour was permanent or related to breeding activities. All animals were eventually released into their original habitats.

Review of museum collections and literature. We examined 2109 museum specimens from two collections, the National Museum of Natural History of the National Academy of Sciences of Ukraine, Kyiv (NMNHU) and the I.I. Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine, Kyiv (SIZK). These specimens were collected from 1983–2017.

We extracted records of colour anomalies from sources published between 1917 and 2017. We only used publications with detailed colour descriptions or with figures that allowed a specific colour assessment. Our reference list is provided in the Appendix. No cases of blue colour in representatives of *R. arvalis* (i.e., completely blue colouration o males during mating season) or *R. temporaria* (i.e., blue colouration of throats in males during mating season; Kuzmin, 2013) were included in the study since such colouration is typical for males of these species during the breeding season (Henle et al., 2017b).

Citizen science. Individual cases of colour anomalies were also obtained from public Facebook groups reporting on Ukrainian fauna. Two groups were monitored for three years, the *Animal World of Ukraine* (https://www.facebook.com/groups/tvarynnyy.svit.ukrayiny) and *Frogs, Lizards and Snakes* (https://www.facebook.com/groups/1449554118682265).

Data analysis. We used QGIS software v. 2.181 (QGIS Development Team, 2016) to georeference the records obtained from the four sources (Tytar et al., 2018). One data point was not included in the study as it could not be georeferenced. Statistical analysis was performed using the package PAST (Hammer et al., 2001).

The number of point localities in Ukraine without the presence of colour anomalies was 1348, which was reduced to 600 by using the nearest neighbour distance method (NTBOX package in R (Osorio-Olvera, 2020) to thin the data, with occurrence points $\leq 0.1°$ apart excluded to avoid spatial autocorrelation.

We selected climatic factors as such that can potentially directly or indirectly influence possible causes (not yet clarified) of colouration anomalies and an increase in the frequency of their manifestation. The values of climate and terrain conditions were extracted from the Global Agro-Ecological Zones database, GAEZ (IIASA and FAO, 2012). Due to the high levels of correlations between the environmental covariates, we filtered the initial variable set of predictors (as listed in the GAEZ database) based on the results of a multi-colinearity analysis. For this purpose, the Variance Inflation Factor (VIF) was calculated using the "usdm" package in R (Naimi et al., 2014), and variables with VIF < 10 were selected as potential explanatory factors for the occurrence of colour anomalies: Terrain Slope Index, Reference Evapotranspiration, Reference Length of Growing Season, Frost-free Period, Annual Precipitation, and Annual Temperature Range (Table 1; see Fischer et al., 2002 for a more detailed account of variables from the GAEZ database). While it is unknown at the present time how some of these factors may influence colour anomalies in amphibians, they all contribute with certainty to the microclimate in any given habitat. In turn, there is documentation regarding the effect of climate conditions on physiological processes in amphibians (e.g., May et al., 2019; Greenberg and Palen, 2021), and microclimate may also act as a key factor in the spread and the persistence of harmful agents that can cause anomalies, including chemicals (e.g., methylmercury; Weiss-Penzias et al., 2019) or organisms (e.g., helminths; Utaaker and Robertson,

Table 1. Selected environmental variables from the Global Agro-Ecological Zones database (IIASA and FAO, 2012) employed during this study.

Variable	Description
Terrain Slope Index Map Unit: Index	This index provides a way to assess the slope of a particular geographic unit for different applications. It is calculated from the different slope classes present in any analysed grid cell.
Reference Evapotranspiration Map Unit: mm	This variable is used to assess the level of evapotranspiration in the habitat of a grid cell, as referenced against green, well-watered grass of uniform height.
Reference Length of Growing Period Map Unit: days	This measure provides a value of habitat productivity in a grid cell based on average climatic parameters.
Frost-free Period Map Unit: days	This number provides an average value for each analysed grid cell of frost-free days during the year with low risk of early and late frosts.
Annual Precipitation Map Unit: mm	This value provides the average annual precipitation expected for the analysed grid cell.
Annual Temperature Range Map Unit: °C	This datum references the difference between the average monthly mean temperature during the months of July and January in a given grid cell.
Human Footprint Map Unit: score, ranging from 0–100.	This data set assesses the human influence on a grid cell and combines population density, land use and infrastructure (e.g., built-up areas, night-time lights), as well as human access (e.g., roads, railroads).

2015). We further used the Human Footprint (HF) data set (Sanderson et al., 2002), a measure of anthropogenic impact, as potential explanatory factor.

Using the QGIS "Point Sampling Tool," raster values of climate, terrain, and HF were matched to the location points of abnormally coloured amphibians and to a background consisting of the 600 absence points. Raster grids of 1 km resolution were used to maximise the extraction of non-duplicate values.

For testing the equality of extracted data means from locations and background points, a Monte Carlo permutation test was employed (Hothorn et al., 2008). This test is non-parametric with few assumptions, and we set the number of permutations to 9999. The power of this test is limited by the sample size; significance at $P < 0.05$ level can be achieved for samples with $n > 3$. For this reason, and because vulnerability to colour abnormalities and distribution ranges could vary across species, statistical analysis was applied only to species and anomalies for which the sample size was ≥ 3.

Results

A total of 130 abnormally coloured individuals were found: 99 during fieldwork, 29 in literature sources, and two cases via citizen science. Most of the colour abnormalities (86.9%; Table 2) were recorded in three species of green frogs, *Pelophylax ridibundus* (57.7%), *P. lessonae* (24.6%), and *P. esculentus* (4.6%), while the rest were recorded in *Lissotriton montandoni* (2.3%), *Bombina variegata* (2.3%), *B. bombina* (1.5%), *Bufo bufo* (3.8%), and *Rana temporaria* (3.1%).

Abnormal colours were found in 13 of 25 oblasts, at 52 locations (Fig. 1). Blue frogs represented 81.5% of all recorded colour anomalies in the genus *Pelophylax* (n = 106), where they occurred exclusively. In 56 individuals, the blue colour was constant and did not fade or disappear with time (Fig. 2A). Presence of these abnormally coloured individuals was confirmed in Nyvky Park (Marushchak et al., 2016), Kyiv City, and Leliaky Village, Poltava Oblast, for two years. This was also the case in one individual that displayed the blue

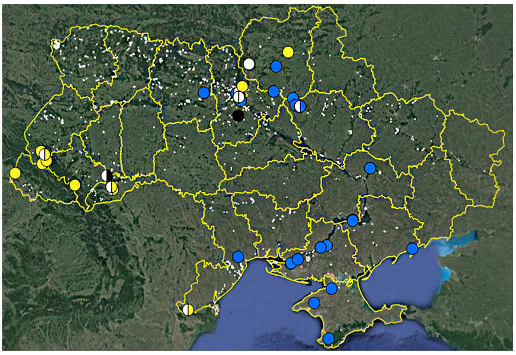

Figure 1. Map of colour anomalies in Ukrainian amphibians. Anomalies are denoted by circles with colours indicating individuals that are fully blue (blue), partially blue (blue/white), leucistic (white), partially leucistic (piebaldism; divided white), fully flavinistic (yellow), partially flavinistic (yellow/white), fully melanistic (black), or partially melanistic (black/white). The small white dots identify locations where no colour anomalies have been recorded.

Table 2. Records of amphibian colour anomalies in Ukraine. Listed sources include records from fieldwork (F), published literature (P), and citizen science (C). An asterisk (*) indicates abnormally coloured individuals, whose abnormal colour reverted to a normal pattern after several days once the animal has been placed in fresh, clean water. Additional abbreviations include nd (no data available), f (female), and m (male).

No.	Species	Description	Year	Latitude (°N)	Longitude (°E)	Sample size	*n*	Sex	Source
1	*P. esculentus*	Blue	2002	50.32	30.59	45	1	juv	F
2	*P. lessonae*	Blue	2005	50.33	32.50	24	1	juv	F
3	*P. lessonae*	Blue*	2015	50.16	32.75	36	1	f	F
4	*P. lessonae*	Blue	2017	50.16	32.75	26	2	juv	F
5	*P. lessonae*	Blue dorsomedial line	2017	50.16	32.75	26	2	f	F
6	*P. lessonae*	Blue dorsomedial line	2017	50.16	32.75	26	5	m	F
7	*P. esculentus*	Blue dorsomedial line	2017	50.16	32.75	5	1	m	F
8	*L. montandoni*	Yellow	1994	48.84	23.31	nd	1	f	P: Grynchyshyn, 2007
9	*L. montandoni*	Yellow dorsum	2006	48.34	24.52	nd	1	f	P: Grynchyshyn, 2007; Smirnov, 2014 a, b
10	*B. bombina*	Orange spot on the head	2009	48.25	25.89	3	1	f	F
11	*P. lessonae*	Black head and other body parts	2011	48.57	25.67	4	1	f	F
12	*B. variegata*	Orange spot behind left eye	2017	48.30	25.84	8	1	f	F
13	*P. ridibundus*	Blue*	2017	50.46	30.40	70	46	nd	F: Fig. 2A, C
14	*P. ridibundus*	Blue	2016	51.05	31.87	nd	1	m	CS
15	*B. variegata*	Cream-yellow	1905	48.92	23.48	nd	1	nd	P: Bayger, 1909
16	*B. variegata*	Orange spot on the back	2007	49.06	23.41	31	1	nd	P: Fedoniuk, 2008
17	*B. bombina*	Black	2013	49.95	30.47	100	1	juv	P: Marushchak and Muravynets, 2018
18	*P. ridibundus*	Blue	2006	45.86	33.88	40	1	f	F
19	*P. ridibundus*	Blue	2006	48.73	35.29	29	1	m	F
20	*P. ridibundus*	Blue	2007	46.83	36.85	32	1	m	F
21	*P. ridibundus*	Blue	2008	45.50	33.26	16	1	m	F
22	*P. ridibundus*	Blue*	2009	47.51	34.66	30	1	m	F
23	*P. ridibundus*	Blue*	2009	47.51	34.66	30	1	m	F
24	*P. ridibundus*	Blue	2010	44.62	33.78	31	1	m	F
25	*P. ridibundus*	Blue dorsomedial line	2010	44.61	33.78	31	1	m	F
26	*P. ridibundus*	Blue	2006	46.47	32.40	11	1	nd	F
27	*P. ridibundus*	Blue	2007	46.48	32.42	33	1	nd	F
28	*P. ridibundus*	Blue dorsomedial line	2006	46.57	32.66	nd	1	nd	F
29	*B. bufo*	Orange spot on the back	2014 2018	50.37	30.50	150	5	m	P: Marushchak and Muravynets, 2018
30	*R. temporaria*	White spot on the back	2013	50.37	30.50	100	1	m	P: Marushchak and Muravynets, 2018
31	*R. temporaria*	Orange spot under the left eye	2013	50.37	30.50	100	1	m	P: Marushchak and Muravynets, 2018
32	*R. temporaria*	Orange spot on the head	2013	50.37	30.50	66	2	m	F
33	*P. ridibundus*	blue on half the body	2001	50.49	30.52	29	1	m	P: Nekrasova, 2002a; Fig. 2B
34	*P. ridibundus*	Blue	2016	50.47	29.21	25	1	nd	F
35	*P. lessonae*	Leucistic	2016	51.11	30.87	nd	1	juv	F: Fig. 2D
36	*P. lessonae*	Blue	2005	50.34	32.48	nd	1	nd	P: Nekrasova, 2014
37	*P. lessonae*	Blue	2014	50.18	32.57	25	2	nd	F
38	*P. lessonae*	Blue	2000	50.15	32.72	27	1	nd	P: Nekrasova, 2014
39	*P. lessonae*	Blue	2010-2011	50.49	31.77	20	10	nd	P: Nekrasova, 2014
40	*P. esculentus*	Blue	2010 2011	50.49	31.77	20	1	nd	P: Nekrasova, 2014
41	*P. ridibundus*	Blue	2010 2011	50.49	31.77	20	1	nd	P: Nekrasova, 2014
42	*P. lessonae*	Blue	2016	50.49	31.78	25	3	nd	F
43	*P. ridibundus*	Blue	2017	46.90	33.71	30	5	m, f	F
44	*P. ridibundus*	Blue	2017	46.85	33.50	21	1	nd	F
45	*P. ridibundus*	Orange spot on the back	2017	45.34	28.66	55	1	juv	F: Fig. 2E
46	*P. esculentus*	Orange spot on the head	2007	48.62	22.33	nd	2	nd	P: Kurtiak and Krulko, 2007
47	*P. lessonae*	Leucistic	1999	51.37	32.31	nd	1	juv	P: Suriadna, 2001

Table 2. Continued.

No.	Species	Description	Year	Latitude (°N)	Longitude (°E)	Sample size	*n*	Sex	Source
48	*P. lessonae*	Blue	2017	50.29	30.57	nd	1	nd	F
49	*P. esculentus*	Leucistic	1913	nd	nd	nd	1	juv	P: Charlemagne, 1917
50	*P. ridibundus*	Blue	2018	46.63	30.47	30	6	nd	F
51	*P. ridibundus*	Yellow	2018	50.60	30.62	nd	1	juv	CS
52	*L. montandoni*	Yellow dorsum	1983	49.14	23.28	14	1	m	P: Smirnov, 2014a, b

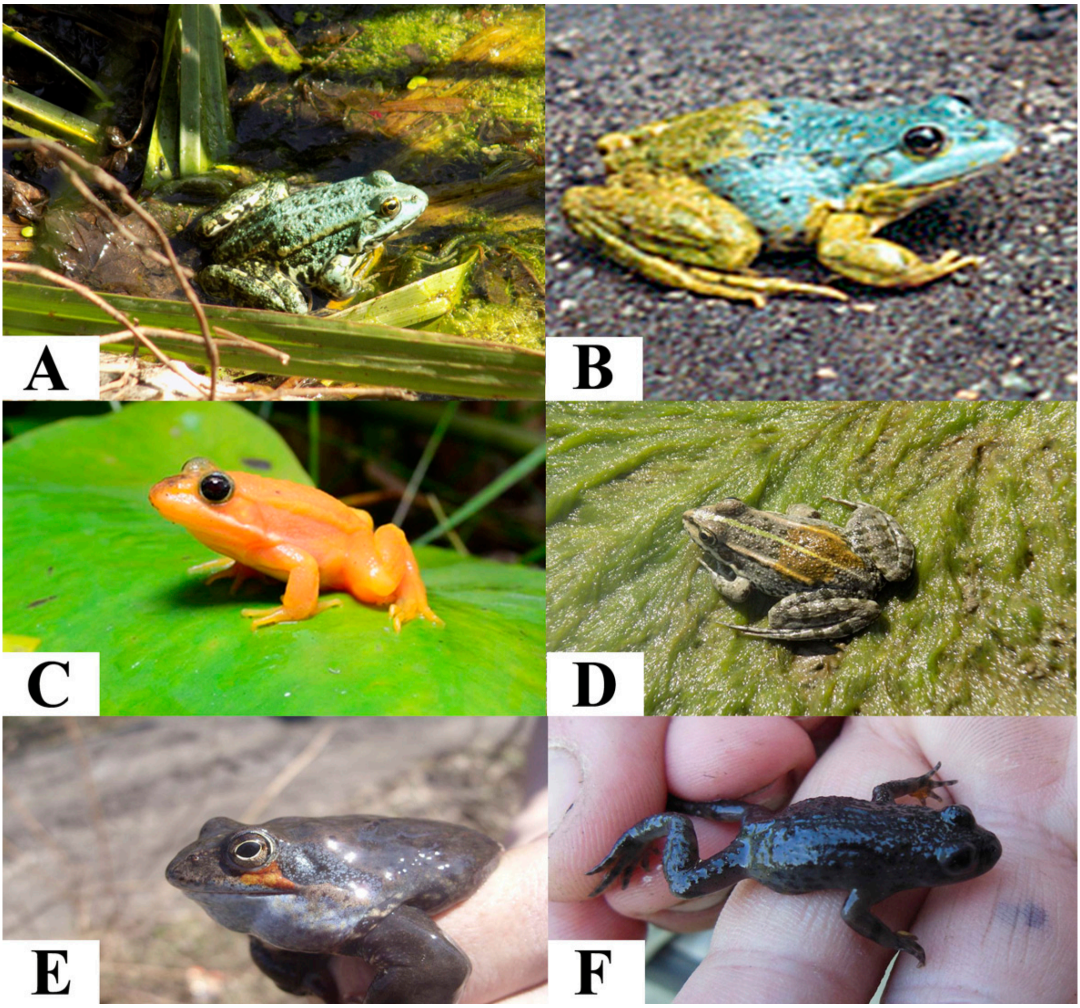

Figure 2. Examples of abnormally coloured Ukrainian frogs. (A) A blue axanthic individual of *Pelophylax ridibundus* from Nyvky Park, Kyiv, photographed by O.Yu. Marushchak in 2016. (B) A partially blue *P. ridibundus* from Verbne Lake, northern Kyiv, photographed by O.D. Nekrasova in 2002 (the photo reduced image quality is due to the use of a contemporary mobile phone). (C) A fully flavinistic *P. lessonae* from Morivsk, Chernihiv Oblast, photographed by O. Lutsenko in 2016. (D) A partially flavinistic *P. ridibundus* from Yalpuh Lake, Odessa Oblast, photographed by O.Yu. Marushchak and O.D. Nekrasova in 2017. (E) An individual of *R. temporaria* from Didorivka Pond, southern Kyiv, photographed by O.Yu. Marushchak in 2013, displaying orange spotting. (F) A fully melanistic *Bombina bombina*, Vasyliv, Kyiv Oblast, photographed by O.Yu. Marushchak in 2014.

colour anomaly only on half of its body (Fig. 2B). In another 49 cases, the blue hue faded and disappeared (Fig. 3) and the animals reverted to a green colour in 3–4 weeks.

Fully flavinistic (yellow) individuals (n = 9; 6.9%; Fig. 2C) were recorded in *L. montandoni*, *B. variegata*, and *Pelophylax* spp. (Bayger, 1909; Charlemagne, 1917; Grynchyshyn, 2007; Kurtyak and Krulko, 2007; Suriadna, 2001; Smirnov, 2014a, b). Partial flavinism or orange spotting (Fedoniuk, 2008) were observed in 11 individuals (8.5%) of *P. ridibundus* (Fig. 2D), *P. esculentus*, *B. bufo*, and *R. temporaria* (Fig. 2E). Only one case of full albinism (0.8%) and one case of leucism in *R. temporaria* (0.8%) were recorded.

Full and partial melanism were observed in two cases, one in *B. bombina* (Fig. 2F) and the other in *P. lessonae*, respectively. The number of locations where colouration anomalies occurred appears to have increased significantly from 1905–2017 (Fig. 4; using logarithmic transformation, $P < 0.05$, Pearson's correlation coefficient = 0.44) as has the number of locations surveyed. Due to lack of information on the number of locations surveyed for the period from 1905 to the 1990s, it is not possible to assess to which extent this increase is due to sampling effort or to a real increase in the prevalence of colour anomalies.

Only for two species and one anomaly type were sample sizes large enough to test differences in the means of environmental variables at locations with and without colour anomalies: blue colouration of the whole body in *P. ridibundus* and *P. lessonae*. Our Monte Carlo permutation test showed a significantly higher Human Footprint at locations with colour anomalies compared to locations where no colour anomalies were found (39.1 vs. 35.7; $P < 0.05$). Reference Evaporation, Length of the Frost-Free Period, Annual Temperature

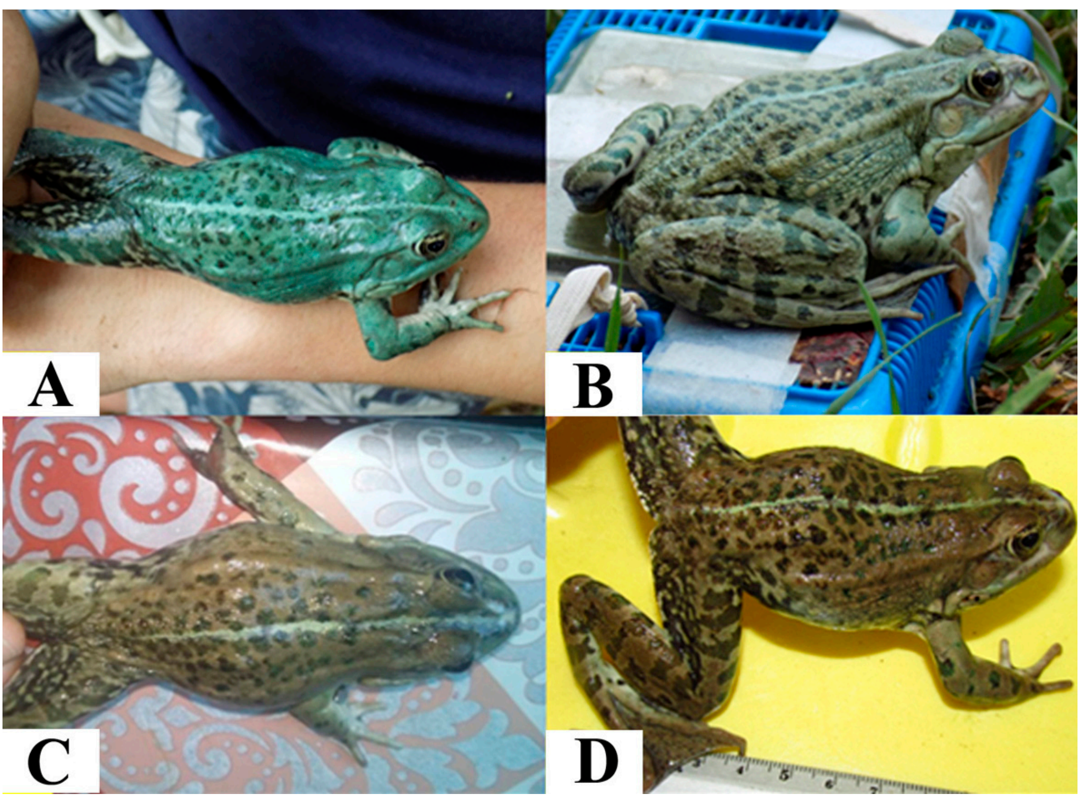

Figure 3. An adult blue individual of *Pelophylax ridibundus*, originally captured in Nyvky Park, Kyiv, photographed in 2016. The sequence of images shows how the abnormal blue colour shifts to the expected green over time. (A) The frog immediately after capture. (B) On Day 4 after capture, a colour shift becomes apparent. (C) On Day 11 after capture, the frog is almost normally coloured. (D) On Day 14 after capture, the frog presents with the expected colouration of an adult marsh frog. Photos by O.Y. Marushchak.

Range, and Human Footprint were higher at locations with blue individuals compared to locations without this type of anomaly, whereas the opposite was the case for Length of the Growing Period and Annual Precipitation (Table 3).

Discussion

There is generally very little information in the literature on colour anomalies in Ukraine. Reports on colouration anomalies are generally included merely as an aside (if at all) in broader investigations (e.g., Nekrasova, 2002c; Fedoniuk, 2008; Smirnov, 2014b) and, consequently, country-specific details may easily be missed by a wider audience. The first colour anomalies were reported for the years 1905 (Bayger, 1909) and 1913 (Charlemagne, 1917). Nearly a century later, colour anomalies are now being more systematically reported from different oblasts of Ukraine (Suriadna, 2001; Nekrasova 2002a–c, 2014; Grynchyshyn, 2007; Kurtyak and Krulko, 2007; Fedoniuk, 2008; Smirnov, 2014a, b; Marushchak and Muravynets, 2018) but these data are still not widely publicised. This is a critical issue, however, since it appears that the number and spectrum of colour anomalies reported have recently increased significantly (Fig. 4; Nekrasova and Kuybida, 2018). Since 2013, a significant manifestation of orange-yellow spots has been recognised in small, isolated populations of *R. temporaria* and *B. bufo* in Kyiv (Table 3, Nos. 29–32). This anomaly has been documented only in recent years but makes up more than 10% of the 52 locations with recorded colour anomalies (Table 3).

In Latvia, in contrast, few colour abnormalities have been registered since 2004 (M. Pupins, pers. comm.) and we know of only three older publications that reported colour anomalies for the country: tadpoles of *Pelophylax ridibundus* (Borkin et al. 1981, 1982; Lusis & Tsaune, 1984). The most abnormal case found in Latvia so far was an extremely dark, probably melanistic individual seen in 2015 in a group of 60 adult *P. lessonae* (Udensrozes, Kraslava District, 55.9326°N, 27.2083°E; M. Pupins, unpubl. data).

Frogs of the *Pelophylax esculentus* complex present the broadest set of colour anomalies, especially in Kyiv, Poltava, and Kherson Oblasts, which have a large number of agricultural enterprises that widely use toxic chemicals, including DDT (historically) and Furadan, without proper control. Abnormal blue colour was the most frequently observed colour anomaly in these species. The above-mentioned toxic chemicals and other pesticides used in agriculture are known to affect and cause changes in colouration of different organisms (amphibians among them) (Noriega and Hayes, 2000; Glennemeier and Denver, 2001; Lifshitz and St. Clair, 2016; Van Meter et al., 2019).

Table 3. Means of selected environmental variables for which there is a statistically significant difference ($P < 0.05$) between locations at which blue *P. ridibundus* and *P. lessonae* occurred and those where no blue frogs were found.

Variable	Anomalies Present		No Anomalies
	P. ridibundus	*P. lessonae*	
Reference evapotranspiration	796.1	–	698.5
Reference length of growing period	138.1	–	180.7
Frost-free period	186.6	–	178
Annual precipitation	521.9	–	596.2
Annual temperature range	–	25.8	24.5
Human Footprint	40.9	–	35.7

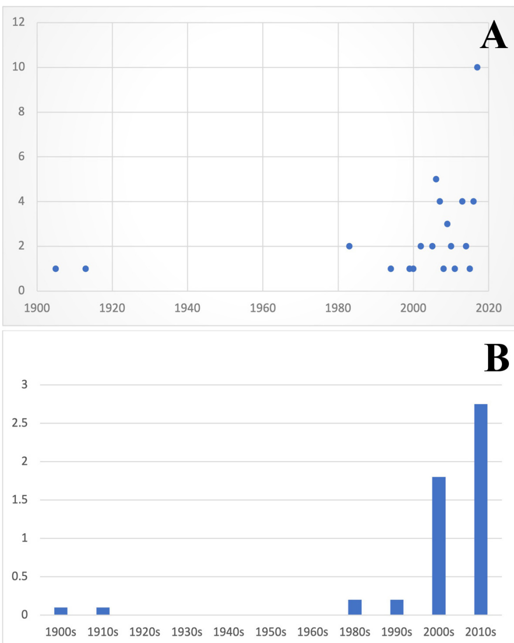

Figure 4. Temporal trends in observations of colour anomalies. (A) Rapid increase in the number of locations where abnormally coloured amphibians were discovered. (B) Average annual encounters by decade. For the period from 1905–2001 our data set includes cases of abnormal colour reported in the literature, whereas for the period from 2002–2017 the data derive from our surveys.

Our records of abnormal blue colour seems to be the first for the *Pelophylax esculentus* complex in the Balto-Black Sea region of Eastern Europe, with no finds of this anomaly reported in nearby countries (e.g., Poland and Latvia). The first incidence was seen by the second author in 2002 in Kozyn Village, Kyiv Oblast. Two additional, unusual cases from the Czech Republic should be mentioned here, an adult female *Ichthyosaura alpestris* with an atypically greyish-blue ventral part of the body and many dots (Dandová and Zavadil, 1993) and an adult blue *P. lessonae* (Vlćek, 2008).

In Ukraine, reports of larger numbers of blue *Pelophylax esculentus* complex frogs (*P. esculentus*, *P. lessonae*, *P. ridibundus*) began to appear during the last two decades. The first cases were recorded in 2001 in Kyiv City (Fig. 2B; Table. 3, No. 33; Nekrasova, 2002a). In 2010–2011 in Zgurivka, Kyiv Oblast (Table 3, Nos. 39–42; Nekrasova, 2014), several blue individuals of this complex were found in cooling ponds of a sugar factory, which still exists. Pollutants dangerous to amphibians, such as formaldehyde and organochlorine compounds (Glennemeier and Denver, 2001; Nekrasova, 2002 a, b, 2014; Van Meter et al., 2019), were found in the pond water.

Unfortunately, very little is known about the causes of abnormal blue colour. As with albinism and leucism, a genetic basis is assumed but, except for the work by Nishioka and Ueda (1985) and Miura (2018) this was never tested. These authors obtained green offspring from crossing experiments involving blue *Dryophytes japonicus* and *Pelophylax nigromaculatus*. The elevated prevalence of blue colour at polluted sampling sites suggests that the anomalies may have been due to a chemically-induced reduction or degeneration of yellow pigment deposition or xanthophore differentiation (Miura, 2018). Our observation that the human footprint was stronger at locations with blue frogs and that about 50% of the blue individuals returned to normal colour within 3–4 weeks when transferred to clean water indicates that pollutants may have disrupted the synthesis or deposition of yellow pigments in the xanthophores in these individuals. The only known similar case of blue colour fading is known for a female *Dryophytes japonicus* from Vladivostok, whose blue colour disappeared after 106 days (Maslova et al., 2018).

Considering the climate-related environmental factors, associations were found mainly for *P. ridibundus* and included both temperature-related variables and annual precipitation. However, at this stage it is difficult to draw any clear conclusions on their causal relevance for the incidence of the observed anomalies. For abnormal black-eyed *Rana arvalis* Vershinin (2004) observed that their frequency was related to low temperature, which possibly may be linked to differential survival of black-eyed individuals. Apart from Vershinin's study there is no other work demonstrating a link between climatic variables and anomalies in amphibians. There is likely a combination of factors in place, where climate change in combination with growing anthropogenic impact on the environment have increased the pressure on amphibians, contributing to a decline in their survival and an increase in the number of anomalies. More systematic studies are required to better understand the relationships of the prevalence of anomalies to climatic stress.

Recently, the number of amphibians and the number of places suitable for their reproduction in Ukraine decreased significantly due to strong anthropogenic impact and climate change (Grebin, 2010; Nekrasova and Kuybida, 2018). Due to the deterioration of water quality, there have been reports of mass mortalities among aquatic organisms (e.g., fish, amphibians) resulting from the eutrophication of water polluted by biogenic elements (Kuybida et al., 2019). In fact, *R. temporaria* has almost completely disappeared from the left bank of the Dnieper River, while *B. bombina*, *P. lessonae*, and *B. bufo* are now extremely rare in the southern part of Ukraine.

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