2018 • vol.16, pp. 76–82
https://doi.org/10.15407/gb.2018.16.076

UDC 597.8(477)

Morphological abnormalities in tailless amphibians (Amphibia, Anura) in Ukraine

O. Y. Marushchak[1], O. A. Muravynets[2]

[1] I. I. Schmalhausen Institute of Zoology, NAS of Ukraine (Kyiv, Ukraine)
[2] Taras Shevchenko National University of Kyiv (Kyiv, Ukraine)

Morphological abnormalities in tailless amphibians (Amphibia, Anura) in Ukraine. — O. Y. Marushchak, O. A. Muravynets. — The present work deals with external morphological anomalies in anuran populations from Ukraine. The work is based on materials collected in 2013–2015, and on specimens from the stock collection of the National Museum of Natural History, NAS of Ukraine (85 specimens from 21 regions). The corresponding maps of Ukraine showing locations with different levels of abnormality were created. Among anurans from Ukraine, a large number of external morphological abnormalities, described in the latest classifications, was discovered. The total percentage of amphibians with anomalies tends to be higher in areas with significant industrial pressure (e.g., Luhansk and Donetsk regions) that could be associated with the combined impact of chemical pollutants, range contraction and fragmentation, and other factors. In addition, several cases of widespread anomalies were found in areas without notable industrial impact (e.g., Zakarpattia). The highest range of malformations was discovered in common toads from the Holosiivskyi district of Kyiv city. A tendency of prevailing of anomalies of traumatic origin was observed as well.

Key words: morphological abnormalities, tailless amphibians, Ukraine, environmental pollution, bioindication.

Introduction

Morphological abnormalities are rather a common issue for almost all populations of amphibians in Ukraine, which is the result of habitat preferences of this group of vertebrates. Inhabiting different habitats during their ontogenesis, these animals suffer from direct and indirect influence of pollutants, mostly of anthropogenic origin, that leads to the development of internal (spine deformation, changes in the morphology of inner organs, etc.) and external (polymelia, ectrodactyly, etc.) morphological abnormalities. Based on their origin, anomalies are divided into several groups such as ontogenetic (occurring because of violated gene expression on the early stages of ontogenesis due to harmful environmental influences), genetic (due to inherited mutations), regenerative (violations during regeneration processes), traumatic (caused by predators), parasitic (provoked by various parasites, e.g., Trematoda), and anomalies caused by illnesses. Finding out the reason of origin of a particular anomaly is very hard, as they usually are phenotypically identical and can be a result of combined effect of several factors (Vershinin, 2015).

Therefore, the aim of the present study was to detect the presence of widespread external morphological anomalies in different amphibian species from the territory of Ukraine.

The appearance of widespread abnormalities is usually induced by pollution of the amphibians' natural habitats (Zamaletdinov, 2003; Kurtyak, 2005; Nekrasova et al., 2007; Borkin et al., 2012; Aguillon-Gutierrez, 2012). The importance of research on links between massive external abnormalities and anthropogenic pollution is widely referred in the literature. Such studies not only rarely include removal of animals from their natural environment, but also allow quickly and clearly show the presence of contamination of a given area with its subsequent localization and elimination. In addition, detecting anthropogenic pressure on populations of amphibians is important in order to provide the respective conservation status to separate populations of these animals. It would have a positive impact on expanding the limits of existing and creation of new protected areas, in anticipation of increasing control over the influence of industry and agriculture on wildlife.

Correspondence to: O. Marushchak; I. I. Schmalhausen Institute of Zoology, NAS of Ukraine, Bohdan Khmelnytsky St. 15, Kyiv, 01601 Ukraine; e-mail: vse_okei@bigmir.net

 p-ISSN 2617-6157 • e-ISSN 2617-6165

The frequency of morphological phenodeviations, as well as the level of fluctuating asymmetry are important to measure the stability of ontogenesis and therefore to monitor amphibian populations under the pressure of various factors. However, it must be mentioned that not only pollution can be responsible for the occurrence of widespread anomalies. Biogeographic factors such as localization at the range edge, presence of non-typical habitats, fragmentation of environment, availability of natural hybridization (hybrid zones) etc. can also cause occurrence of widespread morphological malformations (Vershinin, 1989, 2015).

Material and methods

The material was collected in March–August 2013, April–August 2014, and May 2015 in Kyiv, Rivne, Chernihiv, Cherkasy, Zakarpattia, and Volyn regions of Ukraine by randomized sampling. In addition, data from fixed batrachological materials from the stock collection of the National Museum of Natural History, NAS of Ukraine (NMNH, Kyiv), as well as results of similar studies conducted by other Ukrainian authors (Flyaks, Borkin, 2004; Kurtyak, 2005; Fedonyuk, 2006; Nekrasova et al., 2007) were used (Table 1). The Latin species names are given in accordance with recent nomenclature surveys (Pysanets, 2007; Dubois, Bour, 2010).

Anomalies were considered any deviations from the mean value or parameter, common for a given species, beyond the limits of variation (Prisny, 2009) and noticeable with naked eye. To determine and describe anomalies we used the classification proposed by O. D. Nekrsova (Nekrasova, 2008), as well as the guidelines compiled by Borkin et al. (2012). To estimate the occurrence of malformations we calculated the frequency of occurrence of individuals with abnormalities (P_{as}, %) (the number of

Table 1. **The total number of studied amphibian specimens from different regions of Ukraine**
Таблиця 1. **Загальна кількість досліджених зразків амфібій з різних областей України**

Regions	Species											
	Bombina bombina	*Bombina variegata*	*Bufo bufo*	*Bufotes viridis*	*Hyla orientalis*	*Pelobates fuscus*	*Pelophylax esculentus*	*Pelophylax lessonae*	*Pelophylax ridibundus*	*Rana arvalis*	*Rana dalmatina*	*Rana temporaria*
Cherkasy	—	—	—	—	—	—	—	—	45	—	—	34
Chernihiv	32	—	—	—	—	—	—	96	—	—	—	—
Chernivtsi	—	—	—	—	—	—	—	61	36	—	—	34
Dnipropetrovsk	507	—	—	79	—	—	—	—	395	24	—	—
Donetsk	—	—	—	—	—	—	—	—	110	—	—	—
Ivano-Frankivsk	68	—	—	—	—	—	—	—	—	—	—	26
Kharkiv	—	—	—	—	—	—	29	—	—	—	—	—
Kherson	60	—	—	117	—	—	—	—	23	88	—	—
Kyiv	240	—	300	215	17	65	38	61	65	33	—	145
Luhansk	—	—	—	—	—	—	—	—	93	—	—	—
Lviv	—	31	32	—	35	—	—	—	—	—	—	—
Mykolaiv	—	—	—	38	—	—	—	—	92	—	—	—
Odesa	—	—	—	—	—	—	—	—	97	—	—	—
Poltava	—	—	—	—	—	67	—	—	—	15	—	—
Rivne	—	—	—	50	—	12	—	164	—	156	—	—
Sumy	—	—	—	—	—	—	—	—	34	—	—	—
Vinnytsia	—	—	—	—	—	—	—	—	—	—	—	48
Volyn	88	—	—	—	—	—	—	123	—	27	—	69
Zakarpattia	—	184	—	—	—	—	89	—	46	—	91	49
Zaporizhzhia	—	—	—	—	—	—	—	—	62	—	—	—
Zhytomyr	60	—	—	31	—	30	—	28	—	—	—	—

individuals having various disabilities (N_{as}) to the total individuals in the sample (N), $P_{as}=N_{as}/N$) and the total spectrum of abnormalities (S_{ap}) (the general set of variants of anomalies in a sample). In addition, such parameters were calculated as the partial frequency of occurrence of anomalies (A_{as}, %), which shows the proportion of an anomaly in the sample and in the general population. This parameter is calculated as a proportion of a certain type of anomaly (N_{ab}) to the entire set of morphological malformations in the sample or in the general population (N_{al}) ($A_{as} = (N_{ab}/N_{al}) * 100\%$).

Record localities were marked on the map of Ukraine using Google Earth. When using museum collections or samples of other authors we determined the record locality according to the description on the specimen's label attached by the original. Points showing the frequency of anomalies occurring in samples were displayed on maps of contamination of the territory of Ukraine by different pollutants (http://www.rav.com.ua/).

Results and discussion

In total, 5014 specimens of anurans belonging to 12 species of 8 genera of 5 families were studied and examined for the presence of morphological abnormalities during the period from March 2013 to May 2015. Among them, 344 individuals (6.86 %) were discovered having a single kind or a combination of various malformations. We allocated 409 anomalies likely to be caused by genetic abnormalities and injuries acquired during post-metamorphic stage of the lifespan. In addition, the significant predominance of the number of anomalies over the number of abnormal specimens indicates the wide combinations of malformations (Marushchak, Muravynets, 2013).

As a result of combined investigation of our samples, collection materials, and literature data, widespread anomalies were noted (P_{as} > 5 %) (Borkin et al., 2012) not only in the industrial regions of Dnipropetrovsk (P_{as} = 47.3 %, P_{as} = 33.0 %, P_{as} = 6.3 %, P_{as} = 63.0 %) (Flyaks, Borkin, 2004), Luhansk (P_{as} = 20.0 %), P_{as} = 23.53 %) and Kharkiv (P_{as} = 17.24 %), and in regions of suggested hybridization zones where local appearance of abnormal amphibians is expected such as in Zakarpattia (P_{as} = 66.3 %) (Kurtyak, 2005), but also in the territory of Kyiv (P_{as} = 60.0 %, P_{as} = 42.0 % (Nekrasova et al., 2007), P_{as} = 19.33 %), Zaporizhzhia (P_{as} = 10.0 %), Zhytomyr (P_{as} = 10.71 %), Chernihiv (P_{as} = 10.81 %), Chernivtsi (Pas = 13.11 %), Ivano-Frankivsk (P_{as} = 16.22 %), Cherkasy (P_{as} = 24.0 %) and Volyn (P_{as} = 11.11 %) regions, where anomalies appear locally. The maps of contamination of the territory of Ukraine (https://sites.google.com/site/mygis22/academic-projects, accessed on 18.01.2016; https://bit.ly/2MBb2br, accessed on 18.01.2016) were used as template to shown the distribution of obtained parameters (P_{as}, %) in different regions (Figs 1–3).

Deviations of limbs were found among all of the detected anomalies (see Table 2), including brachydactyly, the shortening of fingers due to the absence of distal phalanges (A_{as} = 41.32 %), ectrodactyly, an anomaly of the "claw," only two fingers, (A_{as} = 1.22 %), oligodactyly, a lack of fingers, which does not form a "claw" (A_{as} = 10.02 %; Fig. 4), abnormal skin folds as fold-like excessive proliferation of the skin of extremities (A_{as} = 0.73 %), hemimelia, forelimb shortening (A_{as} = 0.24 %), ectromelia, hindlimb shortening (A_{as} = 2.20 %; Fig. 6), rotated limb segments as various distorted parts of the limbs at the joints (A_{as} = 2.44 %), polydactyly, appearance of additional fingers (A_{as} = 6.84 %; Fig. 7), polyphalangia, doubled phalanges (A_{as} = 2.44 %), syndactyly, fused fingers (A_{as} = 2.20 %), clinodactyly, curved digits (A_{as} = 4.40 %), phocomelia, absence of forelimbs (A_{as} = 9.05%), amelia, lack of limbs (A_{as} = 2.70 %).

Abnormalities of the head were detected as well, including "crocodile snout," enlargement of the upper jaw (A_{as} = 0.98 %; Fig. 8), eye malformations as blindness, pupil deformity and hypoplasia (A_{as} = 0.73 %; Fig. 4), as well as other forms of morphological distortions, such as anomalies of the skin as its damage, appearance of atypical, differently coloured spots (A_{as} = 7.82 %), wounds and cuts (A_{as} = 1.71 %), wart-like structures (A_{as} = 0.24 %), ulcerative lesions near the resonator (A_{as} = 1.47 %), atypical calluses (A_{as} = 0.73 %) and swelling of parts of the limbs (A_{as} = 0.24 %; Fig. 5).

Table 2. **The partial frequency of occurrence (A_r, %) of external morphological abnormalities in samples with widespread manifestation of the detected anomalies**

Таблиця 2. **Парціальна частота трапляння (A_r, %) зовнішніх морфологічних аномалій у вибірках, у яких було зареєстровано масовий характер прояву аномалій**

Anomaly (A_r, %)	Cherkasy*	Chernihiv*	Chernivtsi*	Ivano-Frankivsk*	Kharkiv*	Kyiv-1*	Kyiv-2*	Luhansk-1*	Luhansk-2*	Volyn*	Zakarpattia*	Zaporizhia*	Zhytomyr*
Abnormal skin formation	—	—	—	—	—	1.6	—	—	—	—	—	—	—
Amelia	—	—	—	—	—	—	—	—	—	—	13.1	—	—
Brachydactilia	50	75	72.7	62.5	91.6	85.3	55.5	75	41.7	33.4	—	25	66.7
Callus	—	—	—	—	—	4.9	—	—	—	—	—	—	—
Damaging of resonators	—	—	—	—	—	—	—	—	—	11.1	—	—	—
Ectrodactyly	—	—	—	—	—	—	2.8	—	—	—	—	25	—
Ectromelia	—	—	—	—	—	1.6	—	—	8.3	—	—	—	—
Eye malformations	16.7	—	—	—	—	—	5.5	—	—	—	—	—	—
Jaw anomaly	—	—	—	—	—	—	2.8	—	—	—	—	—	—
Clinodactyly	—	—	—	—	—	6.6	13.9	25	—	11.1	—	—	—
Limb segment rotation	—	—	—	—	—	—	2.8	—	—	—	—	—	—
Oligodactyly	—	—	—	25	—	—	2.8	—	8.4	—	22.6	50	—
Phocomelia	—	—	—	—	—	—	—	—	—	—	36.9	—	33.3
Polydactyly	—	—	—	—	—	—	—	—	25	—	27.4	—	—
Polyphalangia	—	—	—	12.5	8.4	—	2.8	—	8.3	—	—	—	—
Syndactyly	—	—	27.3	—	—	—	2.8	—	—	—	—	—	—
Skin malformations	16.7	25	—	—	—	—	8.3	—	—	33.3	—	—	—
Wounds and cuts	16.6	—	—	—	—	—	—	—	8.3	11.1	—	—	—

* Note:

Cherkasy — P. ridibundus. Cherkasy region, Kaniv Nature Reserve farmstead, Dnipro riverbank, 2013.
Chernihiv — P. lessonae. Chernihiv region, Ozeriany village, No. A2868, Larionov I.
Chernivtsi — P. lessonae. Chernivtsi region, Storozhynets village, No. A660, 1978.
Ivano-Frankivsk — B. bombina. Ivano-Frankivsk region, Halych town, No. A507, 1978.
Kharkiv — P. esculentus. Kharkiv region, Gaidari village, No. A3378, 2005.
Kyiv-1 — P. fuscus. Kyiv region, Vyshcha Dubechnia village, 2014.
Kyiv-2 — B. bufo. Kyiv city, Didorivka lake, 2014.
Luhansk-1 — P. ridibundus. Luhansk region, Stanytsia Luhanska suburbs, Siverskyi Donets river, No. A1668, 1982.
Luhansk-2 — P. ridibundus. Luhansk region, Stanytsia Luhanska suburbs, Siverskyi Donets river, No. A1422, 1982.
Volyn — P. lessonae. Volyn region, swamp near Svitiaz lake, 2014.
Zakarpattia — P. esculentus. Zakarpattia region, Mynaj village (Kurtyak, 2005).
Zaporizhia — P. ridibundus. Zaporizhia region, Novi Petrivtsi village, No. A64, 1972.
Zhytomyr — P. ridibundus. Zhytomyr region, Vystupovychi village, No. A864.

The highest range of anomalies (S_{ap} = 9) was defined for *B. bufo* sample from the Holosiivskyi district, Kyiv. S_{ap} = 5 was found for *P. fuscus* sample from Vyshcha Dubechnia village (Kyiv region), *P. ridibundus* sample from the Siverskyi Donets river in the suburban area of Stanytsia Luhanska (Luhansk region) and *P. lessonae* from Shatsk town (Volyn region). The vast majority of investigated samples (48/85) had S_{ap} value from 1 to 4.

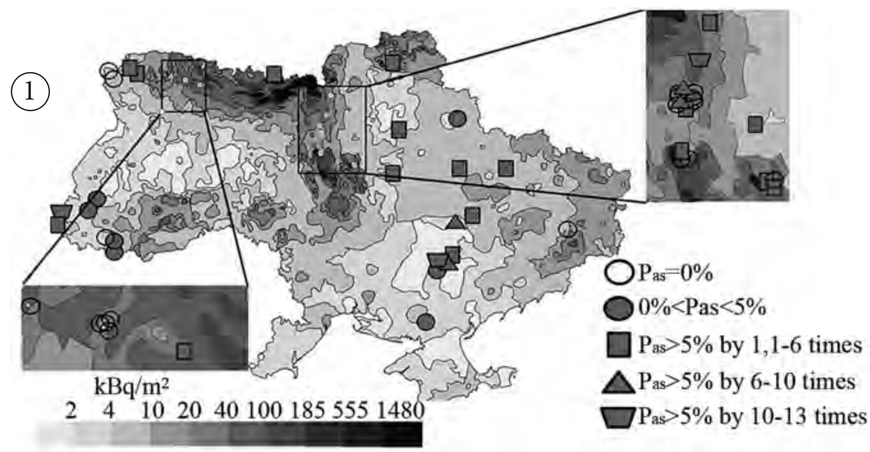

Fig. 1. The map of contamination of the territory of Ukraine with Cs-137 showing the distribution of anomalies in different regions. Only samples since 1986 are shown.

Рис. 1. Мапа забруднення території України з Cs-137 та поширення аномалій в різних регіонах. Показано лише вибірки з 1986 р.

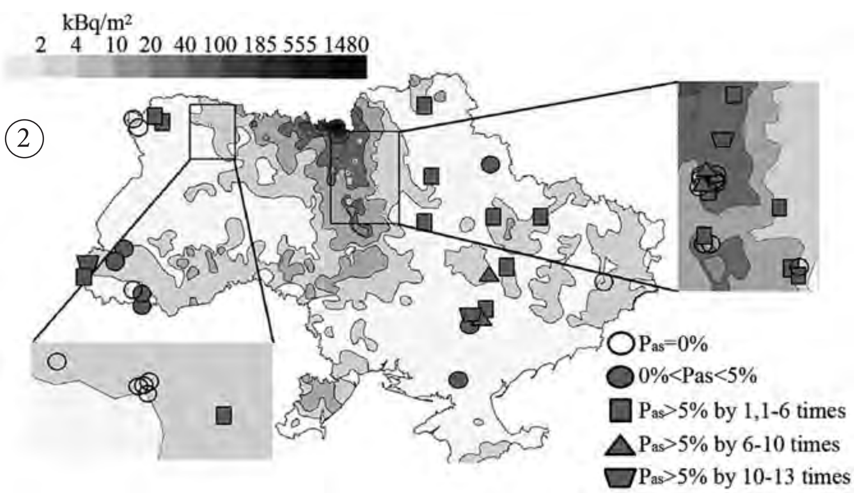

Fig. 2. The map of contamination of the territory of Ukraine with Sr-90 showing the distribution of anomalies in different regions. Only samples since 1986 are shown.

Рис. 2. Мапа забруднення території України Sr-90 та поширення аномалій в різних регіонах. Показано лише вибірки з 1986 р.

Conclusions

A large number of external morphological abnormalities, most of which were described in the latest classifications in CIS (Vershinin, 2015) was found during the research. The discovery of a new anomaly, namely the “crocodile snout” malformation of the upper jaw in the common toad had been confirmed for 3 years in the same population (2013–2015). This anomaly was not described previously in the literature.

The total percentage of amphibians with morphological abnormalities tends to be higher in areas with significant industrial anthropogenic pressure that might be associated not only with chemical pollutants, but with limited spatial isolation of amphibian communities due to range fragmentation, habitat reduction, delineation of their existence, and inbreeding depression (Vershinin, 1989). The highest range of anomalies (S_{ap} = 9) was registered for *B. bufo* sample from the Holosiivskyi district, Kyiv. A tendency of domination of anomalies of traumatic origin (injuries and their consequences) was discovered, which possibly shows not only the direct influence of pollutants causing violation of the ontogenesis hence leading mutations, but also their indirect effect, which leads to greater vulnerability to predators. Instances of locally frequent anomalies were also found in areas that do not feature industrial impact.

Such studies are important and, despite the negative results in this study, in the future, might prove the connection between a certain type of contamination and of morphological abnormalities or their existence as a fact. This will play a significant role in monitoring the state of the environment, bioindication, and species conservation in Ukraine.

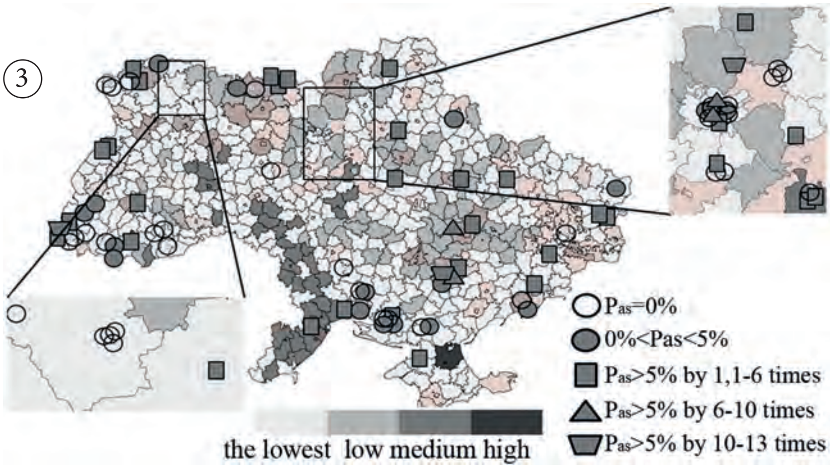

Fig. 3. The map of contamination of the territory of Ukraine with pesticides and heavy metals showing the distribution of anomalies in different regions. Samples before and after 1986 are shown.

Рис. 3. Мапа забруднення території України пестицидами і важкими металами та поширення аномалій в різних регіонах. Показано вибірки як до, так і після 1986 р.

Fig. 4. Pupil deformity, *B. bufo*, male, Didorivka lake, Kyiv.

Рис. 4. Розтікання зіниці у самця *B. bufo*, м. Київ, оз. Дідорівка.

Fig. 5. Swelling of the hip, *R. dalmatina*, female, Kamyanytsia, Uzhhorod district, Zakarpattia region.

Рис. 5. Набряк стегна у самиці *R. dalmatina*, Закарпатська обл., Ужгородський р-н, с. Кам'яниця.

Fig. 6. Ectromelia and oligodactyly, *B. variegata*, male, Mala Uholka, Tiachiv district, Zakarpattia region,

Рис. 6. Ектромелія та олігодактилія у самця *B. variegata*, Закарпатська обл., Тячівський р-н, с. Мала Уголька.

Fig. 7. Symmetrical polydactyly, *P. ridibundus*, female, Siverskyi Donets river, suburb of Stanytsia Luhanska, Luhansk region.

Рис. 7. Симетрична полідактилія у самиці *P. ridibundus*, Луганська обл., р. Сіверський Донець, околиці смт Станиця Луганська.

Fig. 8. "Crocodile snout," malformation of the upper jaw, *B. bufo*, male, Kyiv, Didorivka lake.

Рис. 8. Аномалія щелепи «крокодиляче рило» у самця *B. bufo*, Київ, озеро Дідорівка.

Acknowledgements

Many thanks to V. M. Peskov and O. D. Nekrasova for supervising this work and helping to write the manuscript, to I. M. Kotserzhynska, I. O. Syniavska, N. A. Petrenko, and S. O. Lopariev for guiding field research during the summer practice in Kaniv Nature Reserve, to O. M. Manuilova and A. Y. Maliuk for the opportunity of working with the collections of the National Museum of Natural History, Ukraine.

References

Aguillon-Gutierrez, D. R. 2012. *Investigation of the influence of anthropogenic pollution of the spawning ponds on the post-embryonic development of three species of tailless amphibians.* Abstract of PhD thesis in biology. Moscow, 1–18. (In Russian)

Borkin, L. J., O. S. Bezman-Moseyko, S. N. Litvinchuk. 2012. Evaluation of animal deformity occurrence in natural populations (an example of amphibians). *Proceedings of the Zoological Institute of RAS*, **316** (4): 324–343. (In Russian)

Dubois, A., R. Bour. 2010. The nomenclatural status of the nomina of amphibians and reptiles created by Garsault (1764), with a parsimonious solution to an old nomenclatural problem regarding the genus Bufo (Amphibia, Anura), comments on the taxonomy of this genus, and comments on some nomina created by Laurenti (1768). *Zootaxa*, **2447**: 1–52.

Fedonyuk, O. V. 2006. Amphibians and reptiles, their morphological anomalies and non-standard phenomena as consequence anthropological influences upon forestry ecosystems in Lviv region. *Forestry, Wood, Paper and Wood Industry (Interdepartmental Sci.-Tech. Collection)*, **32**: 355–360. (In Ukrainian)

Flax, N. L., L. J. Borkin. 2004. Morphological abnormalities and heavy metal concentrations in anurans of contaminated areas, eastern Ukraine. *Applied Herpetology*, **1**: 229–264.

Kurtyak, F. F. 2005. Specificity of the population structure of Western-Palearctic green frogs Rana esculenta complex of the flat Transcarpathian. *Scientific Bulletin of Uzhgorod University. Series: Biology*, **16**: 172–175. (In Ukrainian)

Marushchak, O. Yu., O. A. Muravynets. 2013. Morphological abnormalities in natural populations of anurans (Amphibia, Anura) in Ukraine. *Proceeding of the Ukrainian Herpetological Society*, **4**: 87–94. (In Ukrainian)

Nekrasova O. D. 2008. Classification of amphibians anomalies. *Proceeding of the Ukrainian Herpetological Society*, **1**: 55–58. (In Russian)

Nekrasova, O. D., S. V. Mezhzherin, S. Yu. Morozov-Leonov et al. 2007. A case of mass polymelia in the lake frog (Rana ridibunda Pall., 1771) from Kyiv. *Scientific Bulletin of Uzhgorod University. Series: Biology*, **21**: 92–95. (In Russian)

Prisny, Yu. A. 2009. Classification of morphological anomalies of coleopteran insects (Coleoptera). *Scientific Bulletins of Belgorod State University. Series: Natural Sciences*, **11** (9): 72–81. (In Russian)

Pysanets, Y. M. 2007. *The Amphibians of Ukraine*. Rayevsky Publishing House, Kyiv, 1–192. (In Ukrainian)

Vershinin, V. L. 1989. Morphological anomalies of amphibians of city trait. *Ecology*, **3**: 58–66. (In Russian)

Vershinin, V. L. 2015. *Bases of Methodology and Methods of Research of Anomalies and Pathologies of Amphibians*. Publishing House of the Ural University, Ekaterinburg, 1–79. (In Russian)

Zamaletdinov, R. I. 2003. *Ecology of amphibians in the conditions of a large city (on the example of Kazan)*. Abstract of PhD thesis is biology. Institute of Ecology of Natural Systems, Kazan, 1–24. (In Russian)