

**ANOMALIES IN NATURAL POPULATIONS
OF AMPHIBIANS
OVERVIEW, PATTERNS AND CAUSES,
METHODOLOGICAL RECOMMENDATION**

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The article represent analytic review on types and frequencies of amphibian anomalies in more than 2000 natural populations. The review has shown that there are neither standards in the way anomalies are assessed in the field, nor for the way they are scored or reported, which considerably hampers our ability to compare studies. To evaluate temporal trends, and to reveal underlying causes is a field and laboratory experiments that should be designed to test the effect(s) of hypothesized causes of abnormalities.

Статья представляет аналитический обзор о типах и частотах аномалий амфибий в более чем 2 тыс. природных популяций. Обзор свидетельствует, что не существует стандартных способов оценки аномалий в естественных условиях, что значительно затрудняет наши возможности в проведении сравнительных исследований. Для оценки временных тенденций и выявления основных причин необходимы полевые и лабораторные эксперименты, которые должны быть разработаны для проверки гипотетических причин отклонений.

Amphibian anomalies have attracted human curiosity for centuries [e. g., Vallisneri 1706, Geoffroy Saint-Hilaire, 1832–1836; Taruffi, 1881–1886]. Amphibian anomalies also played a pivotal role in the emerging of the science of developmental biology, aiming at understanding morphogenesis [e. g., Hertwig, 1892; Brandt 1924] and they still have this function today [e. g., Kovalenko & Kovalenko 1996; Nye et al. 2003]. Increased environmental awareness, the global decline in amphibians [e. g., Henle & Streit 1990; Alford et al., 2001], and the recent rediscovery in North America of populations exhibiting mass anomalies [Burkhart et al., 2000; Lannoo, 2008; Helgen, 2012] have prompted renewed

interest in amphibian anomalies as potential indicators for environmental perturbations [e. g., Tyler, 1989; Mizgireuv et al., 1984; Souder, 2002; Vershinin, 2002].

Not surprisingly, the literature on amphibian anomalies is enormous. A considerable number of reviews of amphibian anomalies has been published, the first comprehensive one more than a century ago [Taruffi, 1881–1886]. Most reviews focus on narrow topics [Ouellet, 2000] and, with few exceptions (noticeably Rostand 1971), do not discuss to any extent the potential and limits to infer causes from observed patterns of anomalies. Many factors have been shown to cause developmental anomalies in amphibians [Rostand, 1971; Tyler, 1989; Ouellet, 2000; Henle et al., 2014a], but considerable controversy about the causes for observed anomalies in natural populations remains [e. g. Lannoo, 2008; Sessions, 2009; Helgen, 2012]. Linking cause to pattern is crucial for the use of amphibian anomalies as an indicator of the nature of an environmental perturbation that has occurred in the habitat.

The goal of this contribution is to provide a short overview on anomalies in natural populations of amphibians at a global level and particularly for Russia and the Commonwealth States. I will introduce some of the controversies that arose about the causes of malformation hotspots and discuss potentials and limits of inferring cause from patterns of anomalies. Finally, I will make some methodological recommendations for the study of anomalies in natural populations that allow better comparison among studies and that may help elucidate causes for observed anomalies. This publication is an extended summary of parts of a comprehensive review to be published elsewhere [Henle et al., 2014b].

Methods

My overview is based on a collation of 2780 publications collected during the last 30 years that mention abnormal amphibians in natural populations. I extracted relevant information into a database. Often, it is difficult to decide whether a particular phenotype is part of the normal variation, e. g. the high variability in digits of *Salamandrella keyserlingii* [Borkin, 1999] or should be regarded as abnormal (in most species) as this differs among taxa (Henle & Dubois in prep.). I tallied only those forms

as anomalies that were called abnormal by the respective author(s). I included injuries as it is often difficult to differentiate them from teratogenic anomalies (reviewed by [Henle et al., 2014b]).

If a publication contained information on several species, I regarded each species as a separate case. The same applied for several populations of a species, provided sample sizes and anomalies were tallied separately for each population. Otherwise, the data were regarded as one «population». If authors provided data for concrete populations and across sites, I used only data for concrete populations. To allow comparison with Ouellet (2000), I summed data across years and authors for the same population, if the data did not overlap. Otherwise, and in case of unclear overlap (which was the case in several recent North American publications), I used only the data of the most recent publication.

To explore the potential and limitations of inferring cause form patterns of anomalies I reviewed patterns of anomalies obtained in experimental studies from 986 publications. For more information on the methods applied, please consult the extensive reviews of Henle et al. (2014, a, b).

Results and discussion

As of September 2013, the database covers 2239 natural populations for which anomalies have been reported. Most cases involve only one or two individuals (65 % of 1886 cases with data) and background rates are usually around or well below 1 %. Likewise, the vast majority (88 % of 2049 cases with data) involved only one or two types of anomalies. Only four cases from single populations comprise more than 15 types of anomalies. The highest number (32) was exhibited by a population of the green toad (*Bufo viridis*) in Roßwag, Germany, and most likely due to illegally deposited radioactive material [Henle et al., 2014a].

For Russia and the Commonwealth States, I obtained data for 82 populations in which the number of abnormal individuals was tallied. Of these 45 % apply to cases in which only 1–2 individuals were affected (Fig. 1), which is significantly less than for the global dataset ($\chi^2 = 13$; $\alpha < 0,001$). I further obtained data from 99 populations, in which the number of different types of anomalies was tallied. Of these, 63 involved at most two types of anomalies (Fig. 2).

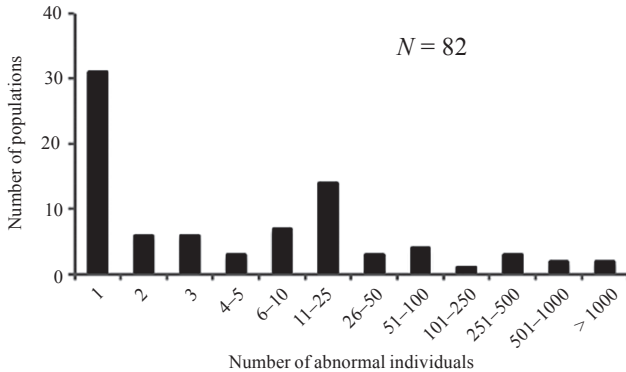


Fig. 1. Histogram of the number of affected abnormal individuals reported from Russia and Commonwealth States

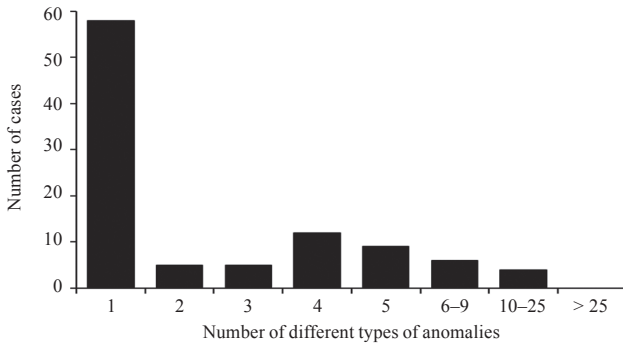


Fig. 2. Histogram of the number of different types of anomalies reported from Russia and Commonwealth States

Again, in Russia and the Commonwealth States a significantly larger percentage of published cases involve a larger number of types of anomalies ($\chi^2 = 47$; $\alpha < 0,001$). There are several mutually not exclusive potential explanations for these differences: a) fewer naturalists and naturalist journals in Russia that publish anecdotal observations on single animals; b) detailed studies are published in journals that are more easily accessible outside Russia; or c) lower water pollution standards combined with a scientific interest in using amphibians as environmental indicators [Pliss & Khudolei, 1979; Vershinin, 2002].

Hotspots of anomalies are known from all continents but especially from North America, Europe, and Asia. In Russia and the Commonwealth States hotspots are known from areas contaminated by sewage or industrial effluents in the Dnepropetrovsk Province, eastern Ukraine [Flax & Borkin, 2004] and from Sakhalin Island [Mizgireuv et al., 1984]. In both cases, the level of organic contamination correlated with the frequencies of anomalies. In 1993, an accident at the Siberian Chemical Combine in Seversk (Tomsk) created another hotspot affecting *S. keyserlingii* and *Rana arvalis* through irradiation [Kuranova, 2003]. In Belorussia, Borkin & Piculik (1986) reported a case of mass polydactyly in *Pelophylax lessonae* that may be a further case of anomaly P. Finally, Woitkewitsch (1965) discovered an enigmatic hotspot in Kazakhstan that affected only *P. ridibundus* but not syntopic ranid species. In all affected individuals the right hind leg was duplicated and other anomalies were rare. The cause for this hotspot remains unresolved.

The evaluation of the experimental literature showed that, contrary to some previous beliefs [e.g. Meteyer et al., 2000; Ouellet 2000], no single type of anomaly is diagnostic for a particular factor, as all types of anomalies can be caused by two or several factors. However, colour anomalies usually, but not always have a genetic basis (reviewed by [Henle et al., 2014b]). Also, the pattern of anomalies induced differs among factors and can be used as indicator for the potential cause(s). For example, a population of *Bufo viridis* from a quarry in Germany exhibited 32 different types of anomalies [Henle et al., 2014a]. Only mutagenic chemicals or radioactivity can cause the range of anomalies observed. Whereas no trace of mutagenic chemicals at elevated concentrations could be found, a high level of radioactivity was found in a deposit of earth.

My extensive review of the literature has shown that there are neither standards in the way anomalies are assessed in the field, nor for the way they are scored or reported, which considerably hampers our ability to compare studies, to evaluate temporal trends, and to reveal underlying causes. For example, Levinskaya & Barinova (1978) provided only the percentage of immature *Rana amurensis* affected by discharge of a cellulose factory (37,5 %) but not sample sizes. However, it is a huge difference whether sample size was 10 or 1000.

In order to draw reliable conclusions about the distribution, frequency, and cause of a given anomaly in a population, data must be gathered at all stages of the study, both in the field and in the laboratory. The field survey protocol should consist in examining carefully all specimens of all ages of all amphibian species collected in the studied population, not only those showing gross anomalies. This will allow having data on the frequency of anomalies, on their distribution among sexes and age classes, and on their variation in time and space. If elevated frequencies of abnormal individuals are discovered, sites should be examined for the presence and association of potential causes of the observed anomalies with the patterns observed in the field. Field and laboratory experiments should be designed to test the effect(s) of hypothesized causes. Dubois & Henle (2014) provide further detailed recommendations for the assessment of individuals in the field and in the laboratory and Lunde & Johnson (2012) guidelines for designing surveys for anomalies in the field. I strongly recommend to follow these guidelines in order to produce more robust and more comparable data on anomalies in future studies.

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Reference

Alford R. A., Dixon P. M., Pechmann J. H. K., 2001. Global amphibian population declines // *Nature*. Vol. 412. P. 499–500.

Borkin L. J., 1999. Salamandrella keyserlingii Dybowski, 1870 – Sibirischer Winkelzahnmolch // *Grossenbacher K., Thiesmeier B.* Handbuch der Reptilien und Amphibien Europas. Schwanzlurche I. Wiesbaden. Aula. P. 21–55.

Borkin L. J., Pikulik M. M., 1986. The occurrence of polymely and polydactyly in natural populations of anurans of the USSR // *Amphibia-Reptilia* Vol. 7. P. 205–216.

Brandt W., 1924. Extremitätentransplantationen an *Triton taeniatus*. Ein experimenteller Beitrag zum Determinationsproblem // *Roux Arch. Entwickl.-Mech.* Vol. 103. P. 517–554.

Burkhart J. G., Ankley G., Carpenter H., Fort D., Gardiner D., Gardner H., Hale R., Helgen J. C., Jepson P., Johnson D., Lannoo M., Lee D., Lary J., Levey R., Magner J., Meteyer C., Shleby M. D., Lucier G., 2000. Strategies for assessing the implications of frog malformations for environmental and human health // *Environmental Health Perspectives*. Vol. 108. P. 83–90.

Dubois A., Henle K. Diversity, terminology, and synonymy of anomalies of amphibians. *Mertensiella*. (in litt.)

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

Geoffroy Saint-Hilaire I., 1832–1836. Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux. P. : Imprimerie de Cosson.

Helgen J., 2012. Peril in the Ponds. Deformed Frogs, Politics, and a Biologist's Quest. Amherst : University of Massachusetts Press.

Henle K., Streit B., 1990. Zum Artenrückgang bei Amphibien und Reptilien und seinen Ursachen // *Verh. Dtsch. Zool. Ges.* Vol. 83. P. 485.

Henle K., Dubois A., Rimpp K., Vershinin V., 2014a. Mass anomalies in green toads (*Bufo viridis*) at Roßwag: inbred hybrids, radioactivity, or mystery cause? // *Mertensiella*.

Henle K., Dubois A., Vershinin V., 2014b. A review of anomalies in natural populations of amphibians and their potential causes // *Mertensiella*. (in litt.).

Hertwig O., 1892. Urmund und Spina bifida // *Arch. mikr. Anat.* Vol. 39. P. 353–499.

Kovalenko E. E., Kovalenko Y. I., 1996. Certain pelvic and sacral anomalies in Anura // *Russ. J. Herpetol.* Vol. 3. P. 172–177.

Kuranova V. N., 2003. Antropiceskoe vosdeistie na zemnovodnysh i presmykayushishtshsja. Problemy ish oshrany na yugo-vostoke Zapadnoi Sibiri // *Amphibii i Reptilii v Zapadnoi Sibiri* / ed. L. N. Erdakov, O. N. Tshernyshtshova. Novosibirsk : Novosibirskii Gosudarstennyi Pedagogitsheskii Universitet. P. 39–51.

Lannoo M., 2008. Malformed Frogs. The Collapse of Aquatic Ecosystems. Berkeley : Univ. California Press.

Levinskaya I. K., Barinova S. S., 1978. On the ecology of the brown frogs of the south of Sakhalin // *Amfibii Dalnego Vostoka i Sibiri* / ed. G. F. Bromlej. Vladivostok : Acad. Nauk. P. 21–23.

Lunde K. B., Johnson P. T. J., 2012. A practical guide for the study of malformed amphibians and their causes // *J. Herpetol.* Vol. 46. P. 429–441.

*Meteyer C. U., Loeffler I. K., Fallon J. F., Converse K. A., Green E., Helgen J. C., Kersten S., Levey R., Eaton-Poole L., Burkhart, J. G., 2000. Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies // Teratology. Vol. 62. P. 151–171.*

Mizgirev I. V., Flax N. L., Borkin L. J., Khudoley V. V., 1984. Dysplastic lesions and abnormalities in amphibians associated with environmental conditions // Neoplasma. Vol. 31. P. 175–181.

Nye H. L. D., Cameron J. A., Chernoff E. A. G., Stocum D. L., 2003. Regeneration of the urodele limb: a review // Developmental Dynamics. Vol. 226. P. 280–294.

Ouellet M., 2000. Amphibian deformities: current state of knowledge // Ecotoxicology of Amphibians and Reptiles / ed. D. W. Sparling, G. Linder, C. Bishop, A. Pensacola. Florida : SETAC Press. P. 617–661.

Pliss G. B., Khudolei V. V., 1979. Oncogenesis and cancerogenous factors in primitive vertebrates and invertebrate animals // Ekologicheskoe Prognozirovanie / ed. V. E. Sokolov. M. : Akad. Nauk. P. 167–185.

Rostand J., 1971. Les étangs à monstres. Histoire d'une recherche (1947–1970). Paris.

Sessions S. K., 2009. Book review: Malformed Frogs, the Collaps of Aquatic Ecosystems by Michael Lannoo 2008 // Herpetol. Rev. Vol. 40. P. 121–124.

Souder W., 2002. A Plague of Frogs. Unraveling an Environmental Mystery. Minneapolis : University of Minnesota Press.

Taruffi C., 1881–1886. Storia della Teratologia. Parte Seconda. Note ed Osservazioni. Fascicolo I–IV. Bologna : Regia Tipografia.

Tyler M. J., 1989. Australian Frogs. Viking O'Neil. Ringwood : Penguin Books Australia Ltd. 220 p.

Vallisneri A., 1706. Galleria di Minerva ovvero notizie universali di quanto e stato scritto da letterati di Europa. Vol. 5. P. 285.

Vershinin V. L., 2002. Ecological specificity and microevolution in amphibian populations in urbanized areas // Advances in Amphibian Research in the Former Soviet Union. Vol. 7. P. 1–161.

Woitkewitsch A. A., 1965. Mass formation of additional hind extremities in the lake frog // Zhurnali obschtschej biologii. Vol. 26. P. 56–62.