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Herpetological methods: II. Protocol for monitoring amphibian deformities under temperate zone conditions

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Abstract. Amphibian deformities have become a more and more common phenomenon in the Northern Hemisphere, and from the early 1990s the frequency of deformity was often above the 2 % background value. In spite of its environmental and conservation importance, only a limited number of studies deal with this topic in Europe. On the basis of a three year national study a suggested protocol to recognise, analyse and evaluate amphibian deformities at the juvenile and adult stage is given in this paper together with some findings on this topic in Hungary. Individual records have value but to analyse amphibian deformity cases further at least fifty individuals per site is suggested to be checked from every species with abundance estimations over at least a 400 m^2 area or a 200 metre riverbank together with the recording of additional biotic and abiotic information.

The rapid destruction of many ecosystem types and the disappearance or serious decline of many species in the last three decades of the 20th century stresses the importance of environmental investigations. Among other programmes, large-scale zoological research projects are needed to understand, conserve and improve the present diversity of species, the stability and functioning of ecosystems on Earth (Purvis & Hector, 2000). Standardisation together with the testing of new methods to develop better sampling protocols is a key element of the process, which is also supported by the present series of articles (Puky, 2001).

Amphibians are among those groups of animals which need extra attention due to their biphasic life cycle, high sensitivity and moderate migrating capacity. Their vulnerability was recognised early (Wake, 1991, Griffiths & Beebee, 1992) and the first standardised protocol was compiled already in 1994 (Heyer et al.). Several publications deals with this topic ever since (see e. g. Olson et al., 1997) as there is a growing need for further improvement, regionalisation or just the contrary, generalisation of monitoring methods.

The occurrence of amphibian deformities is known and documented for centuries as the first description dates back to the 18th century (Vallisneri, 1733). In the last thirty years several authors described and categorised different deformity types and summarised the up-to-date knowledge in this field (Quellet, 1999; Johnson et al., 2001). Apart from genetical reasons, various environmental factors from parasites to low temperature have also been proved to cause deformities in the wild (Dubois, 1983; Gardiner & Hoppe, 1990; Quellet, 1997; Rowe et al., 1996, 1998; Rostand, 1958, 1959, 1971; Woitkewitsch, 1961). Others (e.g. high tadpole density, water chemistry modifications, hot temperature, toxins, lack of vitamin D or calcium) led to malformations under laboratory conditions (Berger, 1968, 1971; Cummings, 1987, 1989; Harfenist et al., 1989; Muto, 1969a, 1969b, 1970). Reports on amphibian deformities have become more common from the early 1990s and the deformity frequency was often well above the 2 % background value including a 71 % frequency of a *Bombina bombina* population in the Gemenc floodplain of the River Danube in Hungary (Puky, 2000). As this phenomenon affects thirty-nine countries worldwide,

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intensive research is needed to elucidate the possible causes and make necessary steps to avoid the conservation consequences of amphibian deformities. To help reach this aim the present paper describes a monitoring protocol suitable for professionals as well as volunteers to recognise, analyse and evaluate amphibian deformities at the juvenile and adult stage together with some findings on this topic in Hungary on the basis of a three year national survey.

METHODOLOGICAL DESCRIPTION

The study of amphibian deformities requires similar licences, preparation and safety precautions as other fieldwork with amphibians (see e. g. Heyer et al., 1994; Olson et al., 1997; Puky, 2001). Here only the specific requirements of amphibian deformity recording are discussed.

Sampling strategies and timing

Unless other factors are taken into consideration, sampling can be carried out most efficiently when animals are most abundant. In the temperate zone this is the breeding season for the adults of terrestrial, fossorial or arboreal species (e.g. *Bufo bufo*, *Pelobates fuscus* and *Hyla arborea*, respectively), which usually peaks in an approximately one to three week period for every species according to the weather conditions. Both spring and autumn migration are optimal periods to check the adults of aquatic or semi-aquatic species in case they hibernate on land. Besides, they can be caught easily in large numbers e.g. when crossing roads as at Lake Fertő (Kárpáti, 1988), and it also means less disturbance to the animals than by other methods. If they hibernate in water, netting or torching (Griffiths & Langton, 1998) can also be used to collect them, but it usually requires more time. For newts, the aquatic period is recommended, which is usually two to four months long and might need different sampling stategies (netting seems to be the best though trapping is also useful when more time is available for sampling). Although involving minimal disturbance, visual examination using binoculars is not an adequate method, as some deformities, e.g. ectrodactyly, are small and often hidden, each individual has to be taken in hand for the investigation. Both because of practical reasons (sampling) and theoretical considerations (deformed animals are more vulnerable to predation, consequently, their ratio decreases over time) newly metamorphosed individuals should be checked as near to metamorphosis as possible. However, if abundance, biomass or any other area-related characteristic is also calculated, sampling should be postponed by at least one, or rather two months to allow juvenile dispersal to take place and thus get better estimations. Checking the water edge or transect sampling are usually good methods for common species while it is often difficult to collect the appropriate number of juveniles from the rare species.

Sample size and area

Records of individuals with deformities even if they describe single animals are useful e.g. to prove the occurrence of a given deformity at a site, in a country or any other geographical unit, or the presence of deformities in a particular species such as the work of Borkin & Pikulik, (1986), Dely (1960), Dubois (1979) and Vershinin (1989). However, to be able to analyse the data further (e.g. to give frequency), several individuals should be checked. Twenty-five individuals is an absolute minimum to be studied, fifty individuals per site is a reasonable number with an optimal number of a hundred individual per site especially if the deformity frequency is over 10 %. Under normal field conditions it is not always possible to collect so many amphibians particularly from rarer species. As a consequence, the number of animals on which the deformity rate is calculated must be included. If less than twenty-five animals can be caught it is still worth recording if they are healthy or not and if e.g out of eight caught frogs two show deformities it is well worth checking the site at another time to get more information, especially as the activity of amphibians greatly changes according to the weather (temperature, moisture, cloud cover, etc.). Usually, more than one species is present at one site even if most are in low numbers; the health condition of rarer species is also of interest, especially when the deformity rate is high

***Table 1.** Short characterisation of amphibian deformity types*

Type of deformity	Short description
Ectrodactyly	Total or partial absence of toe(s)
Ectromely	Total or partial absence of limb(s)
Unilateral anophthalmia	Missing eye
Polyphtalmia	More than two eyes
Syndactyly	Total or partial fusion of toes
Synmely	Total or partial fusion of limbs
Clinodactyly	Curvature of toe(s)
Clinomely	Curvature of limb(s)
Polymely	Supernumerary limb(s)
Polydactyly	Supernumerary toes(s)
Macrophtalmia	Eyes larger than normal
Microphtalmia	Eyes smaller than normal
Subluxatio	Incomplete or partial dislocation of a joint
Tail deformity	Reductive deformity of the tail

in one species. Since disappearance of amphibians can also be a sign of strong negative processes in the environment, the suggested sampling strategy includes abundance estimation over at least a 400 m^2 area or a 200 metre riverbank.

Deformity recording

In amphibians, a highly threatened animal group, deformities may develop in relatively large numbers. When deformities are described, it is important to give detailed information on what deformity type occurred. Table 1 summarises amphibian deformity types according to Quellet (1999). Four important deformity types recorded in Hungary can be seen in Figure 1. In addition to accurate and appropriate categorisation, the indication of the part and side of the body where the malformation occurs can also be relevant. Asconsequence, it is not enough to record e.g. ectrodactyly, but also whether it is on the front or hind legs due to their different development and consequently, the different environmental meaning of the two phenomena. If more than one deformity is present, their symmetry or asymmetry can also be relevant. Figure 2 shows the distribution of leg-related amphibian deformities detected so far in Hungary. Most of them developed on hind legs, which indicates environmental causes (Puky & Fodor, 2002), but front leg deformities and combined front and hind leg deformity cases have also been recorded.

Amphibian deformities have been considered to be mass events if they are present in more than 5 % of the investigated animals (Quellet, 1999). However, our field experience indicates that it is rather the 10 % limit that generally marks the presence of an agent, that affects amphibians. A 2-10 % frequency of amphibian deformities over a longer period, however, can also reveal the presence of an agent that can kill a lot of developing amphibians in an early stage of development (Quellet, 1999). If the size of a popu-

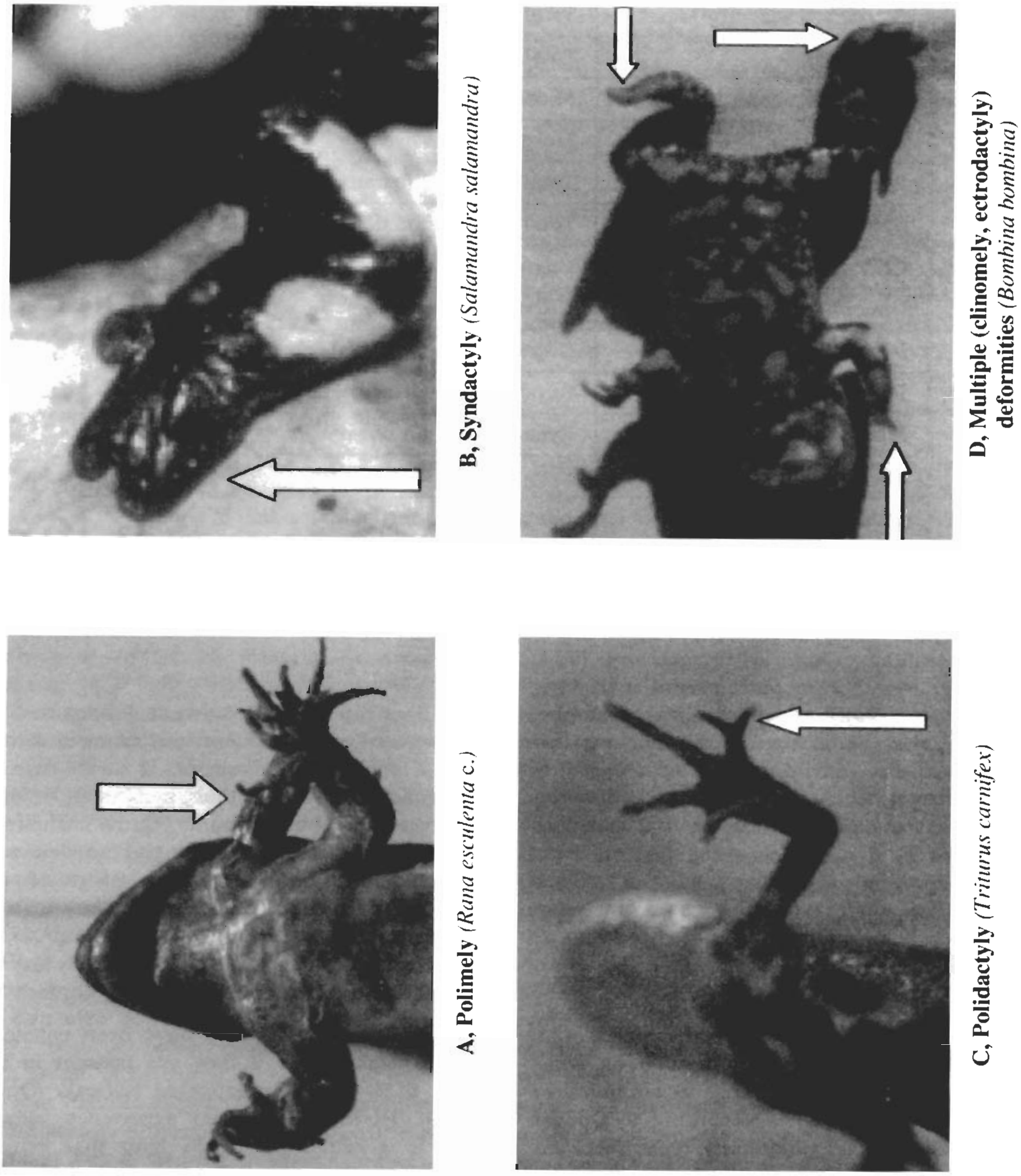

Figure 1. Amphibian deformities in Hungary. A: Polymely (*Rana esculenta* complex); B: Syndactyly (*Salamandra salamandra*); C: Polydactyly (*Triturus carnifex*); D: Multiple (clinomely, ectrodactyly) deformities (*Bombina bombina*)

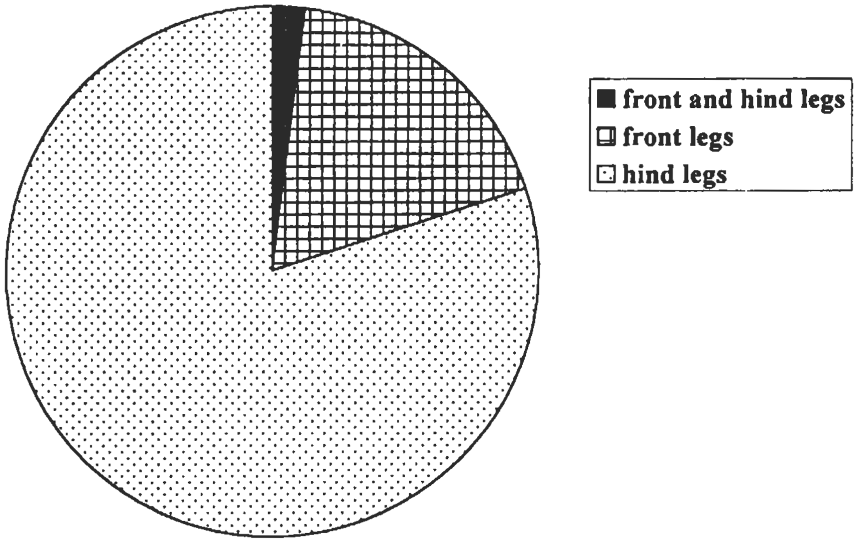

Figure 2. Relative frequency of amphibian deformity types of the front and/or hind legs, recorded in Hungary between 1994 and 2001

lation continuously decreases and also shows a low deformity rate, further studies are urgently needed to find possible causes of this phenomenon before extinction occurs.

Photography can be an important way of making precise records, since the collection of voucher specimens requires an adequate licence. However, if mass occurrence of amphibian deformities is detected or peculiar deformities are found, it is worth contacting nature conservation authorities at once.

Additional information

Additional measurements are strongly encouraged to gain a better understanding of amphibian deformities. Both abiotic (e.g. weather condition, condition of the water monitored, etc.) and biotic information (e.g. body or body and tail length together with weight) are usuful data when the effect of deformities on the population is analysed. Abundance estimation also carries important information on the breeding success and can be in connection with the deformity frequency. The list of possible threats can also be useful in further analysis. Water samples can also be taken but as in most cases water-born pollution is due to a great variety of substances, the usual laboratory analyses may fail to detect the relevant one(s). As a result, water chemistry measurements therefore have a role in more detailed analysis of a site with high deformity rates than in a routine amphibian deformity survey. Even then, negative results are probable reflecting the passage of time before the deformations fully develop.

On the basis of the above considerations, aims and limits, Table 2 summarises the key elements

***Table 2.** Description of key elements of an amphibian deformity survey*

Characteristics	Description or size
Timing	Adults: migration, aquatic period. Juveniles: shortly after metamorphosis.
Method	Species specific, but individuals must be taken in hand, consequently catching by hand, netting or torching are the commonest.
Sampling size	Minimal: 25 individuals/site. Optimal: 100 individuals/site.
Important additional information to collect (abiotic)	Description of the locality (name, geo-cordinates, etc.). Weather conditions. Short description of aquatic habitat. List of possible threats.
Important additional information to collect (biotic)	Short description of vegetation. Biological parameters of the animals (length, weight). Health condition of concommittant species. Presence of other important species (e.g. predators or parasites such as leeches).

***Table 3.** Recordable parameters in an amphibian deformity survey sheet*

Name of locality	
Nearest settlement	
Date	
Recorders	
Weather conditions	Temperature Cloud cover Precipitation Wind
Type of aquatic habitat	Type (e.g. fish pond) Size Vegetation cover
Type of terrestrial vegetation	
Number of species and individuals	Species No. 1. Species No. 2. etc.
Deformity types and numbers per species	
Occurrence of multiple deformities (types, frequency, species)	
Other relevant species	
List of possible threats	
OPTIONAL	
Geo-coordinates	N EO
Water chemistry	
Growth parameters (length, weight of individuals)	

of a successful amphibian deformity survey while Table 3 lists the recordable parameters.

SUMMARY

1. Amphibians are sensitive indicators of environmental changes. One of the reasons is their sophisticated development, which can easily be disturbed by different factors.

2. Reports on amphibian deformities have become more common from the early 1990s and the frequency of deformity was often above the 2 % background value.

3. Amphibian deformities are a multiple cause phenomenon, which needs to be studied in more detail also in the field.

4. On the basis of a three year national study in Hungary, a protocol suitable for professionals as well as volunteers was developed to monitor amphibian deformities.

5. Reports describing individual animals have value, e.g. to prove the occurrence of a given deformity at a site, in a country or any other geographical unit, or the presence of deformities in a particular species.

6. In addition to accurate and appropriate categorisation, the indication of the part and side of the body where the malformation occurs can also be relevant.

7. To be able to analyse amphibian deformity cases further (e.g. to give frequency data) numerous individuals should be checked. Twenty-five individuals is an absolute minimum, fifty individuals per site is a reasonable number with an optimal number of a hundred individual per site especially if the deformity frequency is over 10 %. However, if only a lower number of animals can be caught, it is still worth recording if they are healthy or not.

8. The health condition of rarer species is also of interest especially when the deformity rate is high in one of the common species at the studied site.

9. Further study of populations with deformity rates higher than 10 % is strongly recommended. Abundance estimations over at least a 400 m² area or a 200 metre riverbank proved to be especially helpful.

10. Photography can be an important way of making precise records.

11. If mass occurrence of amphibian deformities is detected or peculiar deformities are found, it is worth contacting nature conservation authorities at once.

12. Traditional water analysis rarely provides relevant information, but the recording of other abiotic or biotic data is important in the future analysis of deformities.

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REFERENCES

BERGER, L. (1968): The effect of inhibitory agents in the development of green-frog tadpoles. *Zool. Polon.*, 18: 381–390.

BERGER, L. (1971): Viability, sex and morphology of F2 generation within forms of *Rana esculenta* complex. *Zool. Polon.*, 21: 345–393.

BORKIN, L. J. & PIKULIK, M. M. (1986): The occurrence of polymely and polydactyly in natural populations of anurans of the USSR. *Amphibia–Reptilia*, 7: 205–216.

CUMMINS, C. P. (1987): Factors influencing the occurrence of limb deformities in common frog tadpoles raised at low pH. *Ann. Soc. R. Zool. Belg.*, 117 (Suppl. 1): 353–364.

CUMMINS, C. P. (1989): Interaction between the effects of pH and density on growth and development in *Rana temporaria* L. tadpoles. *Func. Ecol.*, 3: 45–52.

DUBOIS, A. (1979): Anomalies and mutations in natural populations of the *Rana "esculenta"* complex (Amphibia, Anura). *Mitt. Zool. Mus. Berlin*, 55: 59–87.

DUBOIS, A. (1983): L'anomalie P des grenouille vertes (complexe de *Rana* kl. *esculenta* Linné, 1785) et les anomalies voisines chez les amphibiens. *In: Vago, C.*

& Matz, G. (eds.): Comptes Rendus du Premier Colloque International de Pathologie des Reptiles et des Amphibiens. Angers, France, 215–221.

GRIFFITHS, R. A. & BEEBEE, T. (1992): Decline and fall of the amphibians. *New Scientist,* 1826: 25–29.

GRIFFITHS, R. A. & LANGTON, T. S. (1998): Amphibians: recommended methods. *In: Gent, T. & Gibson, S. (eds.): Herpetofauna Workers' Manual. Peterborough, Joint Nature Conservation Committee,* 34–39.

HARFENIST, A., POWER, T., CLARK, K. L. & PEAKALL, D. B. (1989): A review and evaluation of the amphibian toxicological literature. Ottawa, Ontario, Canada. *CWS. Technical Report Series,* 61: pp. 222.

HEYER, W. R., DONELLY, M. A., MCDIARMID, R. W., HAYEK, L. C. & FOSTER, M. S. (eds.) (1994): Measuring and monitoring biological diversity: Standard methods for amphibians. *Smithsonian Institution Press, Washington,* pp. 364.

GARDINER, D.M. & HOPPE, D. M. (1999): Environmentally induced limb malformations in mink frogs *(Rana septentrionalis). Journ. Exper. Zool.,* 284: 207–216.

JOHNSON, P. T. J., LUNDE, K. B., HAIGHT, R. W., BOWERMAN, J. & BLAUSTEIN A. R. (2001): *Ribeiroia ondatrae* (Trematoda: Digenea) infection induces severe limb malformations in western toads *(Bufo boreas). Canad. Journ. Zool.,* 79: 370–379.

KÁRPÁTI, L. (1988): Massensterben der Herpetofauna (Amphibien und Reptilien) infolge des Kraftverkehrs. Möglichkeiten und Ergebnisse des Schutzes am Südufer des Neusiedlersees. *BFB-Bericht,* 68: 71–79.

MUTO, Y. (1969 a): Anomalies in the hindlimb skeletons of the toad larvae reared at a high temperature. *Cong. Anom.,* 9: 61–73.

MUTO, Y. (1969 b): Hindlimb development and malformations of toes in the larvae reared at high temperature in the toad, *Bufo vulgaris formosus. Cong. Anom.,* 9: 1–12.

MUTO, Y. (1970): Digital malformations in the forelimbs of the toad larvae reared at a high temperature. *Cong. Anom.,* 10: 135–147.

OLSON, D. H., LEONARD, W. P. & BURY, B. R. (1997): Sampling amphibians in lentic habitats: methods and approaches for the Pacific Northwest. Northwest Fauna 4. *Soc. Northwest. Vertebr. Biol.,* pp. 134.

PUKY, M. (2000): Herpetological methods: I. On the use of the road transect method in surveying amphibians with examples from different zoogeographical regions of Hungary. *Opusc. Zool. Budapest,* 32: 75–81.

PUKY, M. (2001): A comprehensive three-year herpetological survey in the Gemenc Region of the Danube–Dráva National Park, Hungary. *Opusc. Zool. Budapest,* 33: 113–128.

PUKY, M. & FODOR, A. (2002): Occurrence of amphibian deformities along the Hungarian section of the River Danube, Tisza and Ipoly. *Limnol. Reports,* 34: 845–852.

PURVIS, A. & HECTOR, A. (2000): Getting the measure of biodiversity. *Nature,* 405: 212–219.

QUELLET, M. (1999): Amphibian deformities: current state of knowledge. *In: Sparling, D. W., Linder, G. & Bishop, C. (eds.): Ecotoxicology of amphibians and reptiles,* 617–661.

ROSTAND, J. (1958): Les anomalies des amphibiens anoures. *Paris, France, SEDES,* pp. 100.

ROSTAND, J. (1959): L'anomalie P chez la grenouille verte (*Rana esculenta* L.). *Bull. Biol. Fr. Belg.,* 93: 7–15.

ROSTAND, J. (1971): Les étangs á monstres: histoire d'une recherche (1949–1970). *Paris, France: Stock,* pp. 85.

ROWE, C. L., KINNEY, O. M. & CONGDON, J. D. (1998): Oral deformities in tadpoles of the bullfrog *(Rana catesbeiana)* caused by conditions in a polluted habitat. *Copeia,* 244–246.

ROWE, C. L., KINNEY, O. M., FIORI, A. P. & CONGDON, J. D. (1996): Oral deformities in tadpoles (*Rana catesbeiana*) associates with coal ash deposition: effects of grazing ability and growth. *Freshwater Biol.,* 36: 723–730.

VALLISNERI, A. (1733): Opere fisico-mediche. Vol. 2. *Venezia, Italy,* pp. 551.

VERSHININ, V. L. (1989): Morphological anomalies in urban amphibians. *Ékologiya,* 3: 58–66.

WAKE, D. B. (1991): Declining amphibian populations. *Science,* 253: 860.

WOITKEWITSCH, A. A. (1961): Le développement des extrémités surnuméraires chez les amphibiens. *Bull. Biol. Fr. Belg.,* 95: 569–600.