Herpetological Conservation and Biology 10(2):679–687.
Submitted: 24 November 2014; Accepted: 3 June 2015; Published: 31 August 2015.

LOW FREQUENCY OF AMPHIBIAN MORPHOLOGICAL ANOMALIES IN A LARGE PROTECTED WETLAND AND GRASSLAND COMPLEX IN HUNGARY

BÉLA MESTER[1], SZABOLCS LENGYEL[1], AND MIKLÓS PUKY[2]

[1]Hungarian Academy of Sciences (HAS), Centre for Ecological Research (CER), Danube Research Institute (DRI), Department of Tisza River Research, 4026 Debrecen, Bem tér 18/c., Hungary,
e-mail: mester.bela@okologia.mta.hu, lengyel.szabolcs@okologia.mta.hu
[2]HAS, CER, DRI, Department of Restoration and Animal Ecology, 1113 Budapest, Karolina út 29., Hungary; deceased

***Abstract.*–The increasing prevalence of morphological anomalies has recently been suspected as a potential mechanism in the global decline of amphibian populations and species. We studied the frequency of morphological anomalies in postmetamorphic amphibians in the Egyek-Pusztakócs marshes (Hortobágy National Park, east Hungary). The site was a wetland and grassland complex used heavily by intensive agriculture until 1973 when it was protected, restored, and managed for biodiversity. We examined 4,953 individuals of 11 species encountered in four monitoring schemes (pitfall traps, bottle traps, dip netting, visual surveys) during 2010–2013. We found no evidence of malformation and a low frequency (0.3%) of abnormalities, which did not differ from the background frequency of 0 to 5% estimated in previous studies for wild populations in natural habitats. All observed abnormalities were found in 15 individuals of four species and were consistent with injuries caused by predators, although the effects of parasites could not be excluded. It remains uncertain whether the absence of malformations and the observed low frequency of abnormalities are related to the 40 years of protection and the long-term decrease in agrochemical use or to the more recent grassland restoration and marsh management actions. Nevertheless, our study provides an example that large, healthy populations of amphibians can exist in large protected wetland complexes restored and managed for biodiversity.**

Key Words.—abnormality; Anura; fertilizer; frog; newt; pollution; toad; Urodela

INTRODUCTION

The global decline of amphibian populations has become an established scientific consensus (Collins and Storfer 2003; Stuart et al. 2004; Hof et al. 2011; Hussain and Pandit 2012). Although reasons for the decline can be related to negative processes in our environment, in particular, to the loss and degradation of freshwater habitats (Dodd and Smith 2003; Cushman 2006), the exact factors and mechanisms of the decline are still not well understood (Allentoft and O'Brien 2010; Blaustein et al. 2011; Pittman et al. 2014). The global decline has also led to increased attention to morphological anomalies due to environmental effects as one potential mechanism of the decline (Boone et al. 2007; Blaustein et al. 2011).

Morphological anomalies in amphibians can be of three types (Johnson et al. 2001): (i) abnormalities, which include any traumatic or developmental gross deviation from the normal range of morphological variation; (ii) malformations, when abnormal development causes permanent structural defects; and (iii) deformities, when an organ or a structure, which was developed correctly, is modified by a mechanical factor (e.g., hind limb amputation). Although the first known observation of a morphological anomaly was reported in the early 18th century (Vallisneri 1733), anomalies have been increasingly studied since the late 1990s (Blaustein et al. 1997; Gardiner and Hoppe 1999; Gray 2000; Blaustein and Johnson 2003). Ouellet (2000) reported anomalies observed in 93 amphibian species worldwide, and at least 24% of these species are found in Europe. Anomalies can occur in association with a variety of factors. Environmental contamination by pesticides (Hayes et al. 2006) or other chemical compounds (Sessions et al. 1999; Hopkins et al. 2000) often result in disruption of the endocrine system, malformations, and/or extra limbs. UV-B radiation reduces survival and increases developmental abnormalities in tadpoles (Pahkala et al. 2001; Ankley et al. 2002). Infection by trematode parasites of the hind limbs was linked to increased limb malformations (Johnson et al. 2002; Blaustein and Johnson 2003). Finally, attempted predation by aquatic predators (e.g., leeches, dragonflies, crayfish, etc.) can lead to injury in the developing limbs, which may resemble malformations in post-metamorphic anurans owing to partial regeneration (Bowerman et al. 2010; Johnson and Bowerman 2010). The frequency of morphological anomalies in a population can reach 80–90% due to these factors and their synergistic or interactive effects (Johnson and Bowerman 2010). In contrast, the endemic (expected) level or background frequency of anomalies was estimated between 0 and 5% for postmetamorphic frogs in wild populations in natural habitats (Vershinin 1989; Ouellet et al. 1997; Piha et al. 2006; Puky 2006; Reeves et al. 2013). For adults, the background frequency is probably much less than 1% (Piha et al. 2006; Puky 2007).

Amphibians have long been known to develop anomalies due to increased exposure to pesticides and nitrogenous fertilizers (Hayes et al. 2006; Brühl et al. 2013). Considering that the use of fertilizers and pesticides is increasing worldwide due to the general intensification of agriculture (Tscharntke et al. 2012), it is reasonable to expect that amphibian decline will further accelerate in regions where they are exposed to these harmful effects. In theory, these effects will be less prevalent in large, undisturbed protected areas, which can thus host healthy populations of amphibians. However, agricultural activities (crop production) are present in a number of European protected areas, and thus amphibians can be exposed to risks of fertilizer and pesticide contamination. Although an evaluation of such risks within Special Areas of Conservation (SACs) established for amphibian species of European conservation importance based on the Habitats Directive of the European Union generally found low risks for globally threatened and European priority species (Wagner et al. 2014), there were several exceptions. For example, the risk of habitat contamination by pesticides was above average for eight species of amphibians (*Pelobates fuscus insubricus*, *Rana latastei*, *Bombina bombina*, *Discoglossus galganoi/jeanneae*, *Triturus dobrogicus*, *T. carnifex*, *T. cristatus*, *T. karelinii*) even in such protected areas, and these species were thus recommended for monitoring the future effects of contamination (Wagner et al. 2014).

The Egyek-Pusztakócs marsh system (EPMS, 4,073 ha) in eastern Hungary (Fig. 1) is part of Hortobágy National Park (established as the first such park in Hungary in 1973) and the Hortobágy SAC (established in 2004). The EPMS has been an active floodplain of rivers Sajó and Tisza since the Pleistocene (Aradi et al. 2003). After river regulation and flood control in the 1860s, the EPMS was gradually drained for agriculture, mainly for crop production. By 1969, croplands covered 35% of the total surface area or 51% of the non-wooded terrestrial habitats, and marshes almost completely dried out (Lengyel et al. 2012). As part of a long-term landscape rehabilitation program, marshes were restored between 1976 and 1997 by the construction of water supply canals (Aradi et al. 2003). The National Park managed the restored marshes using cattle grazing and burning to increase habitat diversity (Mérő et al. 2015). In the next step of the rehabilitation, 760 ha of cropland were restored to grasslands between 2005 and 2008 to decrease the areal proportion of croplands and to protect the marshes and meadows restored previously by establishing ecological corridors between and buffer zones around them (Lengyel et al. 2007, 2012; Mérő et al., In press). Even though the fertilizer and pesticide load on terrestrial and aquatic habitats decreased after the national park was established (1973) and chemical use was limited, it was still relatively high on the croplands before grassland restoration. For example, in 2004, the mean concentration of phosphorus in the soil of the croplands that were later restored was 370 ± 459.0 (S.D.) $mg \cdot kg^{-1}$; whereas, that of potassium was 552 ± 320.2 $mg \cdot kg^{-1}$ (Lengyel et al. 2012). As a result of restoration, the proportion of croplands decreased to 14% of the surface area, grassland corridors and buffer zones were created between marshes and croplands in critical areas, and agricultural pollution and disturbance were reduced in the restored areas (Lengyel et al. 2012).

Given this background, the aim of this study was to survey morphological anomalies in amphibians of the EPMS, especially in *B. bombina* and *T. dobrogicus,* two of the eight species of concern identified by Wagner et al. (2014), which are also among the most common species in the EPMS and tend to show various abnormalities in Hungary (Puky 2000, 2006; Henle et al. 2012). As in other countries, the systematic study of amphibian anomalies was started in Hungary in the early 1990s, and only a few descriptions of additive anomalies were published in Hungary previously (Méhely 1902; Dely 1960). The first systematic surveys on rivers Danube, Tisza, and Ipoly between 1987 and 2001 found anomalies in 13 species (Puky and Fodor 2002; Puky 2006, 2007). However, little information was available on anomalies in lowland wetlands. Thus, a more general aim was to collect information on morphological anomalies from an understudied ecosystem within Hungary.

MATERIALS AND METHODS

The EPMS is a complex of marshes, meadows, alkali and loess grasslands, croplands, and wooded areas (Aradi et al. 2003; Lengyel et al. 2012). The entire area is a Special Protection Area under the European Commission Birds Directive and a Special Area for Conservation under the EC Habitats Directive. Also, a large portion of the park, including the EPMS, is listed as an Important Bird Area, a Ramsar site, and a World Heritage Site (Aradi et al. 2003; Lengyel et al. 2007, 2012). Although no specific herpetological surveys were conducted in the EPMS before, previous descriptions of the fauna of the greater Hortobágy region (Dely 1981; Endes 1988; Puky et al. 2005) mentioned 11 amphibian species that potentially occur in the EPMS: the Common Newt (*Lissotriton vulgaris*), *T. dobrogicus*, *B. bombina*, *P. fuscus*, the Common Toad (*Bufo bufo*), the Green Toad (*Bufotes viridis*), the European Tree

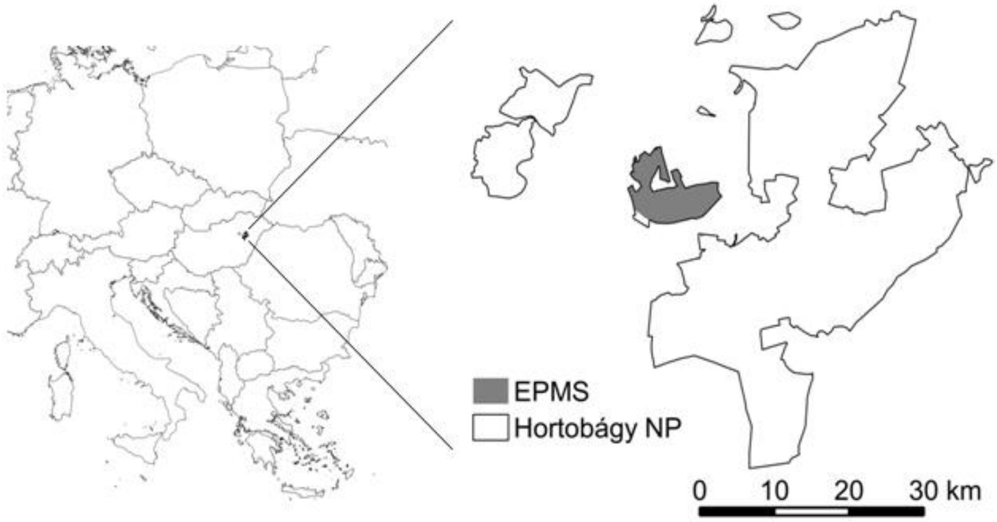


FIGURE 1. Location of Hungary in central Europe, and the Egyek-Pusztakócs marsh system (EPMS) in Hortobágy National Park (NP).

Frog (*Hyla arborea*), the Moor Frog (*Rana arvalis*), and the *Pelophylax* species complex including the Eurasian Marsh Frog (*Pelophylax ridibundus*), the Pool Frog (*Pelophylax lessonae*) and the Edible Frog (*Pelophylax* kl. *esculentus*). All these species are considered typical in the Great Hungarian Plain (Marián 1963; Dely 1967).

As part of a long-term, large-scale landscape rehabilitation program, several conservation interventions were implemented in the EPMS between 2004 and 2013 (grassland restoration, marsh management by burning and grazing, marsh reconstruction). The effects of these interventions on amphibians were followed up in four targeted monitoring schemes between 2010 and 2013. First, in the monitoring of grassland restoration (2010−2013), we caught amphibians using two pitfall traps (0.5 L plastic containers) installed at 45 sites (total n = 90) on 22 fields of restored grasslands over the entire EPMS to sample carabid beetles and ground-dwelling spiders from April to September (Lengyel et al. 2013). Traps contained 100 ml of 25% ethylene-glycol in water with a few drops of detergent as killing liquid, which resulted in the inadvertent death of most amphibians. We checked the traps once in three weeks. Second, a baseline monitoring of four major habitat types along a wet-dry gradient (i.e., marshes, meadows, alkali and loess grasslands), repeated once every three years, consisted of installing two pitfall traps each in 50 habitat patches surrounding Csattag marsh (about 450 ha, 47°35'N, 20°53'E) in the northwest part of the EPMS during the growth season of 2010 and 2013. We also checked these traps once per three week period. Although small (0.5 L) pitfall traps may be more efficient at collecting amphibians of small body size such as juveniles and newts, we occasionally found larger frogs and toads in the traps as well. Third, in the monitoring of marsh management and reconstruction in Fekete-rét marsh (c. 600 ha, 47°33'54"N, 20°55'51"E), we conducted visual and call surveys along 30 randomly selected transects (length: 100 m, width: 4 m) in April 2010 and 2011 (Heyer et al. 1994). During the summer (June/July) of 2011, we used visual encounter and call surveys at the meadows around Fekete-rét and in the dense vegetation of the marsh. Finally, in the dry summer of 2012, when most of the marshes completely dried up, we visually surveyed and installed bottle-traps to sample potential refuge areas that remained wet throughout the summer. Visual and call surveys in these monitoring schemes were of various lengths depending on the area of the habitat patch.

We caught individuals by hand or with a dip net (Dodd et al. 2012). We thoroughly examined all captured postmetamorphic individuals for the presence of morphological anomalies. We identified the type of anomaly using a field guide (Meteyer 2000) and measured the individuals (snout-vent length, body mass). We prepared detailed descriptions, took photographs of the individuals and recorded the coordinates of the locality using a Garmin Dakota 10 handheld GPS (Garmin Ltd., Schaffhausen, Switzerland). We collected individuals found dead in pitfall traps after the examination, and we stored some of them in 70% ethanol for further studies. We immediately removed from the traps the specimens that were still alive, and we

TABLE 1. Number of individual amphibians examined and anomalies found in species detected in the Egyek-Pusztakócs marsh system 2010−2013 with their EC Habitats Directive listing, IUCN conservation status (NT: Near Threatened, LC: Least Concern) and population trend (Temple and Cox 2009). Annex II: species for which special conservation areas are to be designated by the member states; Annex IV: species of community interest, strict protection status. All species are legally protected in Hungary.

Species	EC Habitats Directive Annex		IUCN status and population trend	Number of juveniles/adults examined					Anomaly juv./ad.
	II	IV		2010	2011	2012	2013	Total	
Triturus dobrogicus	X	-	NT, decreasing	2/722	0/12	2/37	0/1	4/772	0/4
Lissotriton vulgaris	-	-	LC, stable	2/69	0/107	4/27	2/0	8/203	–
Bombina bombina	X	X	LC, decreasing	1292/1198	0/50	0/224	0/122	1292/1594	3/3
Pelobates fuscus	-	X	LC, decreasing	0/78	0/71	1/25	0/32	1/206	0/1
Bufo bufo	-	-	LC, stable	5/0	−	−	−	5/0	–
Bufotes viridis	-	X	LC, decreasing	0/7	−	−	−	0/7	–
Hyla arborea	-	X	LC, decreasing	7/3	−	0/21	0/12	7/36	0/1
Rana arvalis	-	X	LC, stable	−	−	0/2	−	0/2	–
Pelophylax complex	-	X*	LC, decreasing	742/0	2/0	34/2	13/23	791/25	3/0
Total				2050/2077	2/240	41/338	15/190	2108/2845	6/9

* *Pelophylax lessonae*

carefully cleaned them and then released them at a safe area nearby after the examination. We released back all other captured individuals at the location of the encounter immediately after the examination.

RESULTS

We caught and examined 4,953 individuals of 11 species (Table 1). Only 15 individuals of five species (11 individuals of four anuran taxa and four individuals of one newt species) showed any morphological anomalies, corresponding to a total frequency of 0.3%. All anomalies were abnormalities and we found no evidence of malformation. In all but one case we observed individuals with complete or partial loss of their hindlimb, forelimb, or tail. The most frequent types of anomaly were ectro- and hemimelia (i.e., the partial or complete loss of the left or the right hindlimb) found in six *B. bombina*, three juvenile *Pelophylax* spp., one adult *H. arborea*, and one adult *P. fuscus*. One of these anurans, a juvenile *B. bombina*, had multiple abnormalities (partial loss of a hindlimb and a forelimb). All limb losses of anurans were asymmetrical. Four *T. dobrogicus* newts showed abnormalities. One subadult had a partial loss of the tail, another had a partial loss of the left hindlimb, and a third one had multiple abnormalities (partial loss of right forelimb and tail). Finally, one *T. dobrogicus* newt had a bifurcated tail, which is described in detail elsewhere (Henle et al. 2012). We did not record mass abnormality (i.e., abnormality frequency above 30%), and the distribution of individuals with abnormalities was sporadic throughout the EPMS.

DISCUSSION

Our surveys detected no malformations and a low frequency (0.3%) of abnormalities in almost 5,000 individuals of amphibian species in a large protected wetland complex. This frequency is far below the high prevalence of morphological anomalies reported in several North American studies (reviewed in Blaustein and Johnson 2003; Sessions 2003) and is at the lower end of the background frequency of amphibian morphological anomalies, estimated between 0 and 5% in previous studies for postmetamorphic anurans (Ouellet et al. 1997; Puky 1999; Vandenlangenberg et al. 2003; Piha et al. 2006; Johnson and Bowerman 2010). These results have conservation relevance especially because the two most abundant species (*B. bombina*, *T. dobrogicus*) of the EPMS are listed as priority species in Annex II of the EC Habitats Directive, and were also predicted by Wagner et al. (2014) to have high risk of pesticide exposure. In addition, all but two of the 11 species detected have a decreasing population trend and are included either in Annex II or IV of the EC Habitats Directive (Temple and Cox 2009). We found large and apparently healthy populations of these and other amphibian species as well, which is relevant because amphibians provide important ecosystem services (e.g., mosquito control) and vital food resources for a number of aquatic birds and birds of prey for which the Hortobágy region is most famous.

Most of the abnormalities occurred in the four most common amphibians of the EPMS: *B. bombina*, and the *Pelophylax* species. This is similar to what Puky (2006) reported based on a study of 50,000 individuals along the rivers Ipoly, Tisza, and Danube in Hungary. *Bufo bombina* and *Pelophyax* species can show numerous types of abnormalities, such as ectromelia, ectrodactyly (missing toe), unilateral anophthalmia (missing eye), syndactyly (total or partial fusion of toes), synmely (total or partial fusion of limbs), clinomely (curvature of limbs), polymely (supernumerary limbs), and polydactyly (supernumerary toes; Puky 2006; Puky and Fodor 2002). Other than ectro- and hemimelia, none of these abnormalities were found in the EPMS. In contrast, only a few observations were previously available on morphological anomalies in *T. dobrogicus* (Puky 2006; Henle et al. 2012), although it is also a common species in Hungary, particularly along the floodplains of rivers Danube and Tisza (Puky et al. 2005). In almost all cases, ectro- and hemimelia were observed in *T. dobrogicus*, which, along with ectrodactyly, is the most frequent type of abnormality in Hungary (Puky 2007). Finally, we detected ectromelia in an adult *H. arborea*, a species in which morphological abnormalities have not yet been reported in Hungary.

Several explanations are possible for the morphological anomalies found in the present study. Because the most frequent anomaly was the loss of hindlimbs, it is possible that anomalies were related to predator attack of juveniles and adults (Bowerman et al. 2010; Johnson and Bowerman 2010). Potential predators include birds (egrets, herons, storks, Kestrel, *Falco tinnunculus*, and Red-footed Falcon, *Falco vespertinus*), the European Pond Terrapin (*Emys orbicularis*), predatory fish, and aquatic arthropods (coleopterans, hemipterans, crayfish). Even though we did not observe signs of injuries (e.g., because injuries to the developing tadpole may not show obvious scarring and wound signs once the animals have metamorphosed), predation is a likely explanation for the anomalies observed. Because we did not observe more severe malformations, such extra limbs, completely missing limbs, or misshapen limbs, it is perhaps less likely that the observed abnormalities could be explained by exposure to retinoid compounds or infection by *Ribeiroia* parasites, which are often the causal agents of such anomalies (Johnson et al. 2002; Gardiner et al. 2003). Because infection of amphibians by Trematoda, Nematoda and Acanthocephala parasites was previously described in the Hortobágy region (Edelényi 1974; Murai et al. 1983), parasites as possible local factors of anomalies cannot be excluded, especially if the effect of parasites is combined with other factors (Reeves et al. 2010; Hof et al. 2011). Finally, because all limb losses of anurans were asymmetrical, there was probably little role of increased UV-B radiation, which typically causes symmetric abnormalities (Pahkala et al. 2001).

Malformations in amphibians are known to occur due to exposure to chemicals used in agriculture such as nitrogenous fertilizers (Marco et al. 1999; Rouse et al. 1999) and pesticides (Hayes et al. 2006). Our results are concordant with those of Piha et al. (2006), who also found low frequencies of deformities in Common Frogs (*Rana temporaria*) in agricultural habitats of Finland and concluded that at the current levels of application, agrochemicals were not a threat to amphibians. Similarly, low frequencies of anomalies were found in Green Frogs (*Rana clamitans*) in southwestern Michigan (Gillilland et al. 2001) and in Japanese Fire-bellied Newt (*Cynops pyrrhogaster*) exposed to agrochemicals (Meyer-Rochow and Asashima 1998). Our findings of no malformations and low frequencies of deformations thus support the conclusions of the above studies that agrochemicals did not represent threats to the morphological development of frogs. Although agrochemicals can still be a threat to amphibians even if few deformities are observed, for example, if their effects are lethal, the lack of dead individuals at our study site showed that this was not likely. It remains uncertain whether the absence of malformations and the low frequency of abnormalities found here reflect improvements in the chemical load due to earlier protection of the area (since 1973) or to the more recent landscape-scale restoration and management actions carried out in the EPMS between 2004 and 2013.

Other factors related to morphological anomalies in Hungary include extremely high tadpole density, high water temperature, and bacterial infection (Puky and Fodor 2002). Such conditions regularly occur in the study area because water levels regularly decrease to a minimum in the EPMS during dry summers. During such environmental bottlenecks, amphibian larvae concentrate in high densities in refuges of permanent water in canals or the deepest parts of the marshes, which warm up quickly and provide good conditions for bacterial infection and increased predation pressure. For example, in summer 2012, which was dry, we found one *T. dobrogicus* individual with an abnormality in a canal that also hosted high numbers of the invasive Amur Sleeper (*Perccottus glenii*) and the native European Weather Loach (*Misgurnus fossilis*). However, mass abnormalities were not observed in this study even in year 2012, which was extremely dry and caused the almost complete drying up of the marshes. Mass abnormalities of amphibians in Hungary were observed only at the Gemenc floodplain after the Danube had flooded the area (Puky 2006, 2007), which shows that it is a rare event. In Hungary, the occurrence of additive morphological anomalies (e.g., polymelia, tail bifurcation, etc.) is also generally rare, with only a few cases known (Dely 1960; Henle et al. 2012). Additive abnormalities can also be linked to injuries based on their rarity and sporadic spatial occurrence in Hungary (Puky 2007).

In conclusion, our four-year monitoring effort found a lack of malformations and a low frequency of abnormalities in a large protected wetland complex with several recent restoration and management actions. All abnormalities could be related to injuries by predation attempts, although the effect of parasites could not be excluded. A full evaluation of whether the observed low frequency is related to the protected status, restoration and management of the study site is not possible due to a lack of previous baseline data. Nevertheless, this study provides an example that large, healthy populations of amphibian species can exist in large protected wetland complexes restored and managed for biodiversity conservation. Further studies should investigate the expected potential causes of abnormalities and their interactions at local and regional scales, focusing on previously determined hotspots (Puky 2007; Reeves et al. 2013).

Acknowledgments.—We dedicate this paper to the memory of Miklós Puky, who passed away during the preparation of the manuscript. We thank Hortobágy National Park Directorate for permitting this study and members of the Conservation Ecology Research Group at the University of Debrecen for help with fieldwork. This research was funded by a grant from the Hungarian Scientific Research Fund (OTKA K106133) to SL. Financial support was provided to BM by the TÁMOP-4.2.4.A/2-11/1-2012-0001 'National Excellence Program, Elaborating and Operating an Inland Student and Researcher Personal Support System', subsidized by the European Union and the State of Hungary and co-financed by the European Social Fund.

Literature Cited

Allentoft, M.E., and J.O'Brien. 2010. Global amphibian declines, loss of genetic diversity and fitness: a review. Diversity 2:47–71.

Ankley, G.T., S.A. Diamond, J.E. Tietge, G.W. Holcombe, K.M. Jensen, D.L. Defoe, and R. Peterson. 2002. Assessment of the risk of solar ultraviolet radiation to amphibians. I. Dose-dependent induction of hindlimb malformations in the Northern Leopard Frog (*Rana pipiens*). Environmental Science & Technology 36:2853–2858.

Aradi, C., S. Gőri, and S. Lengyel. 2003. Az Egyek-Pusztakócsi mocsárrendszer. Pp. 277–298 *In* A Tisza és vízrendszere. Vol. I. Templán, I. (Ed.). MTA Társadalomkutató Központ, Budapest, Hungary.

Blaustein, A.R., and P.T.J. Johnson. 2003. The complexity of deformed amphibians. Frontiers in Ecology and the Environment 1:87–94.

Blaustein, A.R., J.M. Kiesecker, D.P. Chivers, and R.G. Anthony. 1997. Ambient UV-B radiation causes deformities in amphibian embryos. Proceedings of the National Academy of Sciences of the USA 94:13735–13737.

Blaustein, A.R., B.A. Han, R.A. Relyea, P.T.J. Johnson, J.C. Buck, S.S. Gervasil, and L.B. Kats. 2011. The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. Annals of the New York Academy of Sciences 1223:108–119.

Boone, M.D., D. Cowman, C. Davidson, T. Hayes, W. Hopkins, R.A. Relyea, L. Schiesari, and R. Semlitsch. 2007. Evaluating the role of environmental contamination in amphibian population declines. Pp. 32–36 *In* Amphibian Conservation Action Plan. Gascon, C., J.P. Collins, R.D. Moore, D.R. Church, J.E. McKay, and J.R. Mendelson III (Eds.). IUCN/SSC Amphibian Specialist Group, Gland, Switzerland and Cambridge, UK.

Bowerman, J., P.T.J. Johnson, and T. Bowerman. 2010. Sublethal predators and their injured prey: linking aquatic predators and severe limb abnormalities in amphibians. Ecology 91:242–251.

Brühl, C.A., T. Schmidt, S. Pieper, and A. Alscher. 2013. Terrestrial pesticide exposure of amphibians: an underestimated cause of global decline? Scientific Reports 3:1135.

Collins, J.P., and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. Diversity and Distributions 9:89–98.

Cushman, S.A. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biological Conservation 128:231–240.

Dely, O.G. 1960. Une Grenouille Verte (*Rana esculenta* L.) á cinq extrémités – Egy ötlábú kecskebéka (*Rana esculenta* L.). Vertebrata Hungarica 2:41–48.

Dely, O.G. 1967. Kétéltűek – Amphibia. Fauna Hungariae 20:1–80.

Dely, O.G. 1981. Amphibians and reptiles of the Hortobágy. Pp. 387–390 *In* The Fauna of the Hortobágy National Park. Mahunka, S. (Ed.). Akadémiai Kiadó, Budapest, Hungary.

Dodd, C.K., and L.L. Smith. 2003. Habitat destruction and alteration: historical trends and future prospects for amphibians. Pp. 94–112. *In* Amphibian Conservation. Semlitsch, R.D. (Ed.). Smithsonian Institution Press, Washington, D.C., USA.

Dodd, C.K., J. Loman, D. Cogălniceanu, and M. Puky. 2012. Monitoring amphibian populations. Pp. 3577–3635 *In* Conservation and Decline of Amphibians: Ecological Aspects, Effect of Humans and Management. Amphibian Biology XX. Heatwole, H.H., and J.W. Wilkinson. (Eds.). Surrey Beatty & Sons, Chipping Norton, UK.

Edelényi, B. 1974. Mételyek II. Közvetett fejlődésű mételyek. Fauna Hungariae 2:117.

Endes, M. 1988. A hortobágyi hal- kétéltű- és hüllőfauna ökológiai vizsgálata. Calandrella 2:41–56.

Gardiner, D.M., and D.M. Hoppe. 1999. Environmentally induced limb malformations in Mink

Frogs (*Rana septentrionalis*). Journal of Experimental Zoology 284:207–16.

Gardiner, D.M., A. Ndayibagira, F. Grün, and B. Blumberg. 2003. Deformed frogs and environmental retinoids. Pure and Applied Chemistry 75:2263–2273.

Gillilland, C.D., C.L. Summer, M.G. Gillilland, K. Kannan, D.L. Villeneuve, K.K. Coady, P. Muzzall, C. Mehne, and J.P. Giesy. 2001. Organochlorine insecticides, polychlorinated biphenyls, and metals in water, sediment, and green frogs from southwestern Michigan. Chemosphere. 44:327–339.

Gray, R.H. 2000. Historical occurrence of malformations in the Cricket Frog, *Acris crepitans*, in Illinois. Transactions of the Illinois State Academy of Science 93:279–284.

Hayes, T.B., P. Case, S. Chui, D. Chung, C. Haeffele, K. Haston, M. Lee, V.P. Mai, Y. Marjuoa, J. Parker, and M. Tsui. 2006. Pesticide mixtures, endocrine disruption, and amphibian declines: are we underestimating the impact? Environmental Health Perspectives 114:40–50.

Henle, K., B. Mester, S. Lengyel, and M. Puky. 2012. A review of a rare type of anomaly in amphibians, tail duplication and bifurcation, with description of three new cases in European species (*Triturus dobrogicus*, *T. carnifex*, and *Hyla arborea*). Journal of Herpetology 46:451–455.

Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek, and M.S. Foster. 1994. Measuring and Monitoring Biological Diversity, Standard Methods for Amphibians. Smithsonian Institution Press, Washington, DC, USA.

Hof, C., M.B. Araujo, W. Jetz, and C. Rahbek. 2011. Additive threats from pathogens, climate and land-use change for global amphibian diversity. Nature 480:516–519.

Hopkins, W.A., J. Congdon, and J.K. Ray. 2000. Incidence and impact of axial malformations in larval Bullfrogs (*Rana catesbeiana*) developing in sites polluted by a coal-burning power plant. Environmental Toxicology and Chemistry 19:862–868.

Hussain, Q.A., and A.K. Pandit. 2012. Global amphibian declines: a review. International Journal of Biodiversity and Conservation 4:348–357.

Johnson, P.T.J., and J. Bowerman. 2010. Do predators cause frog deformities? The need for an eco-epidemiological approach. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 314:515–518.

Johnson, P.T.J., K.B. Lunde, and E.M. Thurman. 2002. Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. Ecological Monographs 72:151–68.

Johnson, P.T.J., K.B. Lunde, E.G. Ritchie, J.K. Reaser, and A.E. Launer. 2001. Morphological abnormality patterns in a California amphibian community. Herpetologica 57:336–352.

Lengyel, S., S. Gőri, L. Lontay, B. Kiss, I. Sándor, and C. Aradi. 2007. Konzervációbiológia a gyakorlatban, természetvédelmi kezelés és tájrehabilitáció az Egyek-Pusztakócsi LIFE-Nature programban. Természetvédelmi Közlemények 13:127–139.

Lengyel, S., K. Varga, B. Kosztyi, L. Lontay, E. Déri, P. Török, and B. Tóthmérész. 2012. Grassland restoration to conserve landscape-level biodiversity: a synthesis of early results from a large-scale project. Applied Vegetation Science 15:264–276.

Lengyel, S., G. Szabó, B. Kosztyi, B. Mester, T.O. Mérő, P. Török, R. Horváth, T. Magura, I.A. Rácz, and B. Tóthmérész. 2013. Variability in the responses of animal groups to grassland restoration. Pp. 204–209 *In* Dry Grasslands of Europe: Grazing and Ecosystem Services. Vrahnakis, M., A.P. Kyriazopoulos, D. Chouvardas, and G. Fotiadis (Eds.). HERPAS, Prespa, Greece.

Marco, A., C. Quilchano, and A.R. Blaustein. 1999. Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. Environmental Toxicology and Chemistry 18:2836–2839.

Marián, M. 1963. A Közép-Tisza kétéltű és hüllővilága. Móra Ferenc Múzeum évkönyve 1:207–231.

Méhely, L. 1902. A fölösszámú végtagok keletkezéséről. Állattani Közlemények 1:19–34.

Mérő, T.O., L. Lontay, and S. Lengyel. 2015. Habitat management varying in space and time: the effects of grazing and fire management on marshland birds. Journal of Ornithology 156:579-590.

Mérő, T.O., R. Bocz, L. Polyák, G. Horváth, and S. Lengyel. In press. Local habitat management and landscape-scale restoration influence small mammal communities in grasslands. Animal Conservation. doi: 10.1111/acv.12191.

Meteyer, C.U. 2000. Field Guide to Malformations of Frogs and Toads with Radiographic Interpretations. U.S. Geological Survey Biological Science Report USGS/BRD/BSR-2000-0005.

Meyer-Rochow, V.B., and M. Asashima. 1998. Naturally occurring morphological abnormalities in wild populations of the Japanese Newt *Cynops pyrrhogaster* (Salamandridea; Urodela; Amphibia). Zoologischer Anzeiger 221:70–80.

Murai, É., T. Sulgostowska, I. Matskási, F. Mészáros, and K. Molnár. 1983. Parasitic helminths of vertebrates (fishes, amphibians, reptiles and birds) in the Hortobágy National Park. Pp. 15–30 *In* The Fauna of the Hortobágy National Park. Mahunka, S. (Ed.). Akadémiai Kiadó, Budapest, Hungary.

Ouellet, M. 2000. Amphibian deformities: current state of knowledge. Pp. 617–661 *In* Ecotoxicology of Amphibians and Reptiles. Sparling, D.W., G. Linder, and C. Bishop (Eds). Society of Environmental Toxicology and Chemistry Press, Pensacola, Florida, USA.

Ouellet, M., J. Bonin, J. Rodrigue, J.-L. Desgranges, and S. Lair. 1997. Hind limb deformities (ectromely, ectrodactyly) in free living anurans from agricultural habitats. Journal of Wildlife Diseases 33:95–104.

Pahkala, M., A. Laurila, and J. Merilä. 2001. Carry-over effects of ultraviolet-B radiation on larval fitness in *Rana temporaria*. Proceedings of the Royal Society B 268:1699–1706.

Piha, H., M. Pekkonen, and J. Merilä. 2006. Morphological abnormalities in amphibians in agricultural habitats: A case study of the Common Frog *Rana temporaria*. Copeia 2006:810–817.

Pittman, S.E., M.S. Osbourn, and R.D. Semlitsch. 2014. Movement ecology of amphibians: a missing component for understanding population declines. Biological Conservation 169:44–53.

Puky, M. 1999. A kétéltűfauna helyzete és kétéltű fejlődési rendellenességek előfordulása a Körös-Maros Nemzeti Parkban. A Puszta 16:84–100.

Puky, M. 2000. A comprehensive three-year herpetological survey in the Gemenc Region of the Duna-Dráva National Park, Hungary. Opuscula Zoologica 32:113–128.

Puky, M. 2006. Amphibian deformity frequency and monitoring methodology in Hungary. FrogLog 74:3–4.

Puky, M. 2007. Kétéltű- és hüllőkutatás a Magyar Dunakutató Állomáson. Pp. 97–107 *In* A Dunának, mely múlt, jelen s jövendő.... 50 éves az MTA Magyar Dunakutató Állomása (1957-2007). Szemelvények az Állomás tudományos eredményeiből. Nosek, J., and N. Oertel (Eds.). MTA ÖBKI MDÁ, Göd and Vácrátót, Hungary.

Puky, M., and Fodor, A. 2002. Entwicklungsanomalien bei Amphibien in der ungarischen Abschnitten der Donau, Theiss and Eipel. Occurrence of amphibian deformities along the Hungarian section of the River Danube, Tisza and Ipoly. Donau Aktuell 6:2–3.

Puky, M., P. Schád, and G. Szövényi. 2005. Magyarország Herpetológiai Atlasza. Herpetological Atlas of Hungary. Varangy Akciócsoport Egyesület, Budapest, Hungary.

Reeves, M.K., P. Jensen, C.L. Dolph, M. Holyoak, and K.A. Trust. 2010. Multiple stressors and the cause of amphibian abnormalities. Ecological Monographs 80:423–440.

Reeves, M.K., K. Medley, A. Pinkney, M. Holyoak, P.T.J. Johnson, and M. Lannoo. 2013. Localized hotspots drive continental geography of abnormal amphibians. PLoS ONE 8: e77467.

Rouse, J.D., C.A. Bishop, and J. Struger. 1999. Nitrogen pollution: an assessment of its threat to amphibian survival. Environmental Health Perspectives 107:799–803.

Sessions, S.K. 2003. What is causing deformed amphibians? Pp. 168–186 *In* Amphibian Conservation. Semlitsch, R.D. (Ed.). Smithsonian Institution Press, Washington, D.C., USA.

Sessions, S.K., R.A. Franssen, and V.L. Horner. 1999. Morphological clues from multilegged frogs: are retinoids to blame? Science 284:800–802.

Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman, and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Temple, H.J., and N.A. Cox. 2009. European Red List of Amphibians. Office for Official Publications of the European Communities, Cambridge, UK.

Tscharntke, T., Y. Clough, T.C. Wanger, L. Jackson, I. Motzke, I. Perfecto, J. Vandermeer, and A. Whitbread. 2012. Global food security, biodiversity conservation and the future of agricultural intensification. Conservation Biology 151:53–59.

Vallisneri, A. 1733. Opere Fisico-mediche. Vol. 2. Appresso Sebastiano Coleti, Venice, Italy.

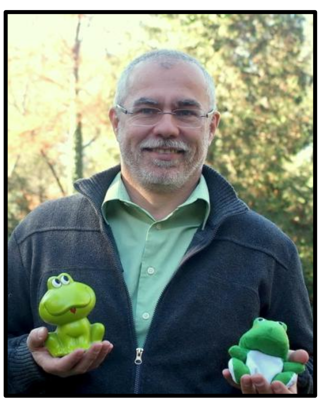
Vandenlangenberg, S., J. Canfield, and J. Magner. 2003. A regional survey of malformed frogs in Minnesota (USA). Environmental Monitoring and Assessment 82:45–61.

Vershinin, V.L. 1989. Morphological anomalies in urban amphibians. Ékologiya 3: 58–66.

Wagner, N., D. Rödder, C.A. Brühl, M. Veith, P.P. Lenhardt, and S. Lötters. 2014. Evaluating the risk of pesticide exposure for amphibian species listed in Annex II of the European Union Habitats Directive. Biological Conservation 176:64–70.

BÉLA MESTER is a Young Researcher at the Danube Research Institute of the Center for Ecological Research of the Hungarian Academy of Sciences. He received his B.S. in Biology in 2009 and an M.S. in Hydrobiology in 2011 at the University of Debrecen, Hungary. He is currently working on his Ph.D. dissertation on the effects of restoration and management on amphibians in an alkali marsh and grassland system. He is an active member of the Toad Action Group Association, BirdLife Hungary, and the Green Circle - Friends of the Earth's member Association. His expertise is in amphibian faunistics, ecology and conservation, aquatic ecology, wetland conservation, and ornithology. (Photographed by László Polyák).

SZABOLCS LENGYEL is a Scientific Advisor (Professor) at the Danube Research Institute of the Center for Ecological Research of the Hungarian Academy of Sciences. He received his Ph.D. in Ecology, Evolution, and Conservation Biology from the University of Nevada, Reno, USA, with Lewis W. Oring and C. Richard Tracy and was a Postdoctoral Scholar at North Carolina State University, USA, with Rob R. Dunn. His Ph.D. dissertation was in behavioral ecology, specifically, on the costs and benefits of chick adoption behavior in shorebirds. He has published over 70 peer-reviewed articles and book chapters, which received more than 1300 citations. His current research focuses on the long-term impacts of habitat restoration and management on animal communities and on the emergence of landscape-level patterns in biodiversity, using the largest active grassland restoration in Europe as a model, which he coordinated in Hortobágy National Park (east Hungary). (Photographed by Balázs Vági).



MIKLÓS PUKY was a Senior Research Fellow at the Danube Research Institute (DRI) of the Center for Ecological Research of the Hungarian Academy of Sciences until he passed away in March 2015. After he received his M.S. in Biology and English translation at Eötvös University in Budapest in 1986, he worked at the DRI. He received his university doctorate in 1992 and his Ph.D. in 2005 from Eötvös University. Miklós had more than 110 publications on the ecology and conservation of amphibians, reptiles, and decapod crustaceans, which received over 680 citations. Miklós was very active both internationally and in non-governmental organizations. (Photographed by Toad Action Group).