COMMENTARY AND PERSPECTIVE

Explanations for Deformed Frogs: Plenty of Research Left to Do (a Response to Skelly and Benard)

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ABSTRACT

Our recent study (Ballengeé and Sessions, 2009. J Exp Zool (Mol Dev Evol) 312B:1–10) shows that deformed frogs with missing limbs can be explained by sublethal "selective predation" by predators that are too small, or have mouthparts that are too small, to consume whole tadpoles. Skelly and Benard do not agree with our conclusions and feel that they are not well founded. Here we respond to their critique. *J. Exp. Zool. (Mol. Dev. Evol.) 314B:341–346, 2010.* © 2010 Wiley-Liss, Inc.

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Our recent article (Ballengée and Sessions, 2009) is the first publication to present experimental evidence supporting the idea that missing limbs in deformed amphibians can be caused by a specific predator: in this case, dragonfly nymphs. These insect larvae (and certain other predators with mouth parts that are too small to consume an entire tadpole) use sublethal "selective predation," attacking or capturing tadpoles and gnawing off their protruding hind limbs, often causing permanent limb deformities in frogs that survive to metamorphosis. Earlier research established that deformed frogs featuring extra limbs are caused by a parasite, specifically the trematode *Ribeiroia ondatrae* (Sessions and Ruth, '90; Johnson et al., '99; Sessions et al., '99; Stopper et al., 2002). With this study, we think we have identified the *proximate causes* for the remaining deformed amphibian mystery: deformed frogs with missing limbs. Thus, we conclude that selective predation, together with parasite infection, accounts for the vast majority of reported deformed frogs, at least those involving limb deformities.

Skelly and Benard disagree with our conclusions, but their critique contains distortions and inaccuracies that require response. First, they claim, or imply, that not enough prior research has been conducted to elucidate any definitive cause for deformed amphibians. Although we agree that the causes for missing limbs have been elusive (which was the motivation for our study), we find their statement that amphibian deformities remain poorly understood "despite more than a decade of research" completely unfounded. In truth, study after study including numerous articles, book chapters, and two books (Souder, 2000; Lannoo, 2008), have been produced over the last decade on this subject. More than 40 articles and book chapters devoted to this topic have been published just from our lab and Pieter Johnson's lab alone, all confirming the role of parasites in the induction of supernumerary limbs in amphibians (Johnson et al., '99, 2001, 2004; Sessions et al., '99; Stopper et al., 2002; Blaustein and Johnson, 2003; Sessions, 2003, to name a few). We think this is a pretty good track record, by any standard. On the other hand, numerous articles have also been published reporting results of investigations into possible links between chemical pollution and these same deformities, with negative or, at best, equivocal results. There is still not a single chemical pollutant that has been shown to actually cause any of the observed kinds of limb deformities in natural populations of amphibians (Degitz et al., 2003; Ankley et al., 2004). Perhaps it would have been more accurate if Skelly and Benard had instead said that published research *supporting the direct role of chemical pollution* is woefully lacking, a sentiment with which we would wholeheartedly agree, especially considering that millions of dollars of research funding have been poured into the effort.

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Skelly and Benard also challenge our use of experimental simulations to better understand natural scenarios. The advantages and disadvantages of laboratory vs. field experiments in ecology are well-known (Brower and Zar, '84; Hairston, '89), and we are aware of the necessary trade-offs. Our approach is grounded in one of the fundamental concepts of research biology—that organisms often display natural behaviors in captive environments (sometimes the only way that they can be observed), and observing this behavior can provide the basis for further understanding their behavior in natural conditions. Also, our experiments were focused not on whether dragonfly nymphs predate tadpoles in the context of complex natural ecosystems (there is plenty of research already showing that they do), *but whether they produce injuries in tadpoles that result in permanent deformities*. This is very similar to the recent experiments done by numerous researchers (Johnson et al., '99; Stopper et al., 2002; Schotthoefer et al., 2003) showing that trematode cercariae induce permanent limb deformities in tadpoles when exposed in isolated containers. Although Skelly (2002) has challenged this approach, it is still a standard practice in biology, and such simulations are widely utilized in the amphibian research community (e.g. captive-breeding programs, pathogen research, genetic research, etc.). Laboratory simulations have been widely used to study arthropod predation of anuran larvae prey (Wassersug, '73; Heyer et al., '75; Brodie et al., '78; Peckarsky, '82; Crump, '84; Formanowicz, '84; Manteifel and Reshetnikov, 2002). Criticism by Skelly and Benard on this point also seems to us to be somewhat hypocritical, as Skelly himself has utilized laboratory experiments to investigate interactions between anuran larvae and their odonate predators (Skelly and Werner, '90).

Skelly and Benard also criticize our study because we did not provide a quantitative analysis of the association between limb deformities and predator densities, even though that was not the focus of our research. They go on to point out that their own observations show no clear relationship between rates of limb deformities in frogs and the distribution and prevalence of dragonfly nymphs. Based on their surveys of very large numbers of frogs from dozens of ponds over several years, they claim to have found extremely low rates of deformities despite an abundance of dragonfly nymphs. For example, out of 36,151 specimens representing eight anuran species, only 10 deformed frogs (0.03%) were found. Likewise, in a sample of 22,482 Wood frogs (*Rana sylvatica*), they found only 15 individuals (0.07%) that were deformed. (Our first reaction to these miniscule rates was "What deformed frogs?") Their reports are at odds with the published research on deformed amphibians. A perusal of multiple surveys of natural populations of frogs over the last 30 years representing nearly 98,000 examined frogs, show rates of deformities ranging from 0.14 to 26.7% and averaging 4.6% (Table 1). Furthermore, in a summary of a decade of research on deformed amphibians, Lannoo (2008) documents that deformities have been reported in 51 species, representing 11 genera and 50% of all US species of anurans, and 19 species representing 6 genera and 10% of US species of urodeles. Field data for three of the most frequently affected species of anurans show average rates of limb deformities ranging from 6.8% in western toads (mean of 1.1 deformities per animal) to 11.9% in Pacific treefrogs (1.4 deformities per animal) to 16.7% in several species of Ranids (1.2 deformities per animal; Lannoo, 2008).

What could explain these disparities in reported incidences of deformities, especially in the association between the frequency of dragonfly nymphs and deformities? First, many researchers tend to focus on "hotspots," i.e. sites with deformity rates of at least 5% (Meteyer et al., 2000; Lannoo, 2008), whereas Skelly and Benard cite studies that apparently represent more general surveys of amphibian populations. These, therefore, may not be relevant to the issue of frequencies of deformed amphibians. Although the long-term research cited by Skelly and Benard may be helpful for general baseline studies of frog populations, we are concerned that they may have used somewhat different methods of data collection.

One of the most fundamental lessons of deformed frog research is that little can be learned from examining metamorphosed frogs (e.g. via drift fences) representing a single "snapshot" of time. Because tadpoles with deformities caused by parasites (e.g. extra limbs) are largely immobile and do not survive long once they metamorphose and lose their tails, this kind of deformity is actually almost never seen except via serendipity. And, because missing limbs are caused by injuries to tadpoles resulting in a range of healing responses from complete regeneration to permanent deformities, depending on the developmental stage of the tadpole (Sessions, '97, 2003; Ballengeé and Sessions, 2009), it is only by monitoring tadpoles (including the same *individual* tadpoles) over time and examining newly metamorphosed individuals that an accurate picture of what is going on can be developed. It is little wonder that deformed amphibians have gone largely unexplained for so long, despite centuries of published reports of isolated incidences.

Assuming their surveys of 58,633 frogs included examinations that were detailed enough to generate an accurate assessment of limb deformities (see Ouellet et al., '97), the fact that Skelly and Benard found only 25 frogs (0.04%) with deformities despite populations of dragonfly nymphs attaining a density of more than $1/m^2$ truly does point to the need for more research. Our recent work (Ballengeé and Sessions, 2009) shows that dragonfly nymphs can and do cause limb injuries that result in a range of permanent deformities that match the range of deformities found in collected anurans. As we point out in our article, the astonishing thing is not that they do this, but that there are not more reports of deformed amphibians. Perhaps tadpoles are not the preferred prey (especially given their skin toxins), so that nymph-induced limb deformities only occur at

Table 1. Summary of recent surveys of deformed amphibians (anurans unless otherwise indicated).

Author(s)	Year(s) of study	Region	No. species[a]	No. examined	No. deformed	% deformed
Ouellet et al., '97	1992–1993	Southern Quebec	4	1,124	108	9.6
Bonin et al., '97	1993	Southern Quebec	1	410	22	5.4
Burkhart et al., '98	1997	Minnesota	1	2,254	199	8.8
Hoppe, 2000	1996–1997	Minnesota	1	2,584	58	2.3
Canfield et al., 2000	1997–1999	Minnesota	1	13,859	902	6.5
Gray, 2000	1998	Illinois	1	140	3	2.1
Levey et al., 2003	1996–2001	Vermont	1	>10,000	~600	6.0
Gillilland et al., 2002	1990–2001	Michigan	6 (4)	2,835	4	0.14
Schoff et al., 2003	1998–2000	Minnesota, Wisconsin, Illinois	7	2,605	42	1.6
McCallum and Trauth, 2003	1990–2000	Arkansas	1	976	79	8.1
Vandenlangenberg et al., 2003	1997–2000	Minnesota	1	More than 5,100	~403	7.9
Eaton et al., 2004	1995–1999/ 2000–2002	Alberta, Saskatchewan	1	21,050	150	0.71
Hoppe, 2005	1996–1999	Minnesota	7	796	19[a]	2.4
Hoppe, 2005	1996–1999	Minnesota	1	2,828	756	26.7
Taylor et al., 2005	2002	Vermont	6	5,264	83	1.6
Skelly et al., 2007	2002–2003	Vermont	7	3,420	117	3.4
Reeves et al., 2008	2000–2006	Alaska	1	9,268	576	6.2
Bowerman et al., 2009	1988–2008	Oregon	1	13,443	343	2.6
Total				97,956	4,464	4.6

Data from field seasons 1988–2008 from recent articles.
[a]Anuran species unless otherwise indicated (caudate species in parentheses).

sites where the preferred prey is absent. Our study lays the foundation for future research focused on this and other unanswered questions.

Skelly's research program is focused on the effects of chemical pollution on amphibians (www.cbc.yale.edu/people/skelly/), and the implications of his recent publications on deformed frogs is that neither parasites nor predation are sufficient to account for all deformed amphibians (Skelly et al., 2007). This leaves chemical pollution as the most likely alternative. Although we agree that chemical pollution is a threat to amphibians and other organisms (and we applaud Skelly's research efforts on that front), current evidence suggests that chemical pollutants are at best only indirectly involved in deformed amphibians of the kind we have been studying (Rohr et al., 2008). The direct role of chemical pollution as a proximate cause of limb deformities in wild amphibians is simply not supported by current evidence (Sessions et al., '99; Ankley et al., 2004). This conclusion is reinforced by examination of the frogs themselves, which usually contain important clues about the proximate causes (Sessions et al., '99; Stopper et al., 2002). First, the abnormalities occur primarily in the hind limbs in otherwise healthy-looking frogs. We might expect the reverse if chemicals were directly altering limb development as anuran forelimbs develop in the tadpole gill chamber and would be exposed to constant flow-through of any dissolved chemical pollutants. What we have found instead, in the case of frogs with extra limbs, are frogs that are often riddled with parasitic (trematode) cysts, especially in and around the limb deformities (though not always, as some frogs may lose these cysts as they develop; Stopper et al., 2002). The extra limbs are mostly duplicated structures characterized by mirror-image symmetry (Sessions et al., '99). Mirror-image symmetry in duplicated limb structures is a tell-tale sign of intercalation, a powerful developmental pattern forming mechanism triggered by the disruption of the spatial relationships of signaling cells in developing limbs (French et al., '76; Bryant et al., '81; Sessions et al., '99; Stopper et al., 2002). The senior author first described this parasite-extra limb deformity link almost 20 years ago (Sessions and Ruth, '90)!

Sessions and Ruth ('90) was the first study to establish a definitive link between trematode parasites and extra-limb

deformities in wild-caught amphibians. This relationship between parasite and host was further established by Johnson et al. ('99) who induced the entire range of deformities by exposing Pacific treefrog tadpoles to trematode cercariae in laboratory simulations. Sessions et al. ('99) identified the trematode as a species of the genus *Ribeiroia* (later confirmed as *R. ondatrae* by Johnson et al., 2001). Similar work by Stopper et al. (2002) elucidated the precise developmental mechanisms involved, confirming the role of intercalation. All these studies have pieced together the fascinating life cycle of a heretofore little understood trematode. In this life cycle, amphibians serve as the second intermediate host and the limb deformities seem to be an example of a host-modification adaptation for which digenetic trematodes are well known. This is now a familiar and widely accepted story, summarized in a recent issue of *Scientific American* (Blaustein and Johnson, 2003).

Solving the mystery of extra limbs was a significant achievement, but it left the largest category of deformed frogs (those with missing limbs) unsolved. Contrary to the assertion by Skelly and Benard, this problem was pointed out by one of us more than a decade ago (Sessions, '97, 2003). Although not as dramatic looking as frogs with multiple extra limbs (which, unfortunately, became the "poster child" of the "malformed frog" issue early on; e.g. Lannoo, '98; Souder, 2002), limbless frogs turned out to be much more difficult to explain. Again, the frogs themselves contain important clues: the vast majority feature abnormalities in the hind limbs (and sometimes adjacent pelvic structures) in the otherwise robust, healthy looking, metamorphosed frogs. Often, they exhibit little to no obvious sign of earlier trauma (e.g. scars, exposed bone, inflammation, reddening) on the affected limb structures, although sometimes, abnormal cartilaginous or other soft-tissue structures and/or abnormal pigmentation are found at the ends of limb stumps (Lannoo, 2008). Although these structures may look "abnormal," there is little to no evidence to suggest that these are outside the range of normal regenerative response to mechanical perturbation of anuran limbs. As it turns out, the answer to the mystery of deformed frogs with missing limbs has been in the historic literature the whole time! Numerous studies have elucidated the broad range of limb deformities associated with injuries incurred at varied stages of development (e.g. Schotté and Harland, '43; Forsyth, '46; Fry, '66; Scadding, '81). Yet, precisely because of the healing ability of anuran amphibians, earlier injuries may be missed because they often lack mammalian-like scars, prolonged reddening, inflammation, exposed bone(s), etc. and this has led many authors to dismiss or downplay predator-induced injury as the cause of the abnormalities (Meteyer et al., 2000; Lannoo et al., 2003; Skelly et al., 2007; Lannoo, 2008). The absence of evidence of trauma combined with the well-known regenerative decline that occurs with normal tadpole limb development (Muneoka et al., '86), readily accounts for the range of peculiar, idiosyncratic deformities found in the wild. Even among our test animals in carefully controlled conditions, we found a range of abnormal-looking regenerative limbs identical to those we have observed in the field over the past 10 years of research (Ballengeé and Sessions, 2009).

Our research sought to find the types of predators that could induce injuries resulting in missing limb deformities. Our article (Ballengeé and Sessions, 2009) is the first publication to explicitly link a known amphibian predator (dragonfly nymphs) to missing limb deformities in amphibians through carefully controlled experiments. Odonate larvae are known feeding specialists with a wide-ranging assortment of foraging behaviors correlated with adaptive morphologies (for review see Corbet, '99). Some authors have rejected predation-induced injury as a potential cause of deformed frogs with missing limbs because, they argue, it is difficult to imagine how the predators would not either fully consume whole tadpoles or induce lethal injury (e.g. Lannoo et al., 2003, 2008). This position ignores an important group of anuran larval predators including arthropods, annelids (leeches), some fishes, and even tadpoles themselves that partially consume their tadpole prey (Formanowicz, '84; Veith and Viertel, '93; Sessions, '97, 2003; Manteifel and Reshetnikov, 2002). Selective predation (sometimes referred to as "sublethal" or "predatory grazing") is a well-known ecological phenomenon practiced by a variety of predators generally attacking similar sized prey (Sih, '80). The results of our predator/prey experiments again confirmed our hypothesis: dragonfly nymphs can injure tadpoles resulting in metamorphic anurans with permanent limb deformities identical to those we observed in the field. We think we have provided strong experimental and field evidence in support of selective predation as a critical piece of the "deformed frog puzzle."

We stand by our conclusion that the results of our research provide an important contribution to understanding the origin of deformities in amphibians; in this case, the most common reported deformities, missing limbs and limb segments. We think that our research provides the basis for future work and we anticipate that additional findings supporting our work will soon be forthcoming. Our conclusion is that most hind limb deformities in wild-caught anurans (i.e. missing limbs) are the result of natural regenerative responses to traumatic injuries from selective predation and, in the case of extra limbs, parasitic infection. The amazing thing about deformed amphibians with extra limbs is that it took about a decade to confirm what we already knew 20 years ago: that the extra limbs are caused by trematode infection. It is remarkable that it has taken so long to identify a convincing cause for missing limbs. Nevertheless, as with parasite-induced deformities, there are still many unanswered questions, and we agree with Skelly and Benard that there is much more research to be done.

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LITERATURE CITED

Ankley GT, Degitz SJ, Diamond SA, Tietge JE. 2004. Assessment of environmental stressors potentially responsible for malformations in North American anuran amphibians. Ecotoxicol Environ Saf 58:7–16.

Ballengeé B, Sessions SK. 2009. Explanation for missing limbs in deformed amphibians. J Exp Zool (Mol Dev Evol) 312B:1–10.

Blaustein AR, Johnson PTJ. 2003. Explaining deformed amphibians. Sci Am 2003:60–65.

Bonin J, Ouellet M, Rodrigue J, DesGranges JL, Gagné F, Sharbel TF, Lowcock LA. 1997. Measuring the health of frogs in agricultural habitats subjected to pesticides. In: Green DM, editor. Amphibians in decline: Canadian studies of a global problem. Herpetological conservation, number 1. St. Louis, MO: Society for the Study of Amphibians and Reptiles. p 258–270.

Bowerman J, Johnson PTJ, Bowerman T. 2009. Sublethal predators and their injured prey: linking aquatic predators and severe limb abnormalities in amphibians. Ecology 91:242–251.

Brodie Jr ED, Formanowicz Jr DR, Brodie ED. 1978. The development of noxiousness of *Bufo americanus* tadpoles to aquatic insect predators. Herpetologica 34:302–306.

Brower JE, Zar JH. 1984. Field methods and laboratory methods for general ecology, 2nd edition. Dubuque, IA: Brown Publishers.

Bryant S, French V, Bryant P. 1981. Distal regeneration and symmetry. Science 212:993–1002.

Burkhart JG, Helgen JC, Fort D, Gallagher K, Bowers D, Propst TL, Gernes M, Magner J, Shelby MD, Lucier G. 1998. Induction of mortality and malformation in *Xenopus laevis* embryos by water sources associated with field frog deformities. Environ Health Perspect 106:841–848.

Canfield JT, Kersten SM, Vanselow P. 2000. 1997–1999 Field Season Report. Minnesota Malformed Frog. Investigation, Minnesota Pollution Contral Agency, Minneapolis, MN. 75p.

Corbet PS. 1999. Dragonflies: behavior and ecology of Odonata. Ithaca, NY: Cornell University Press.

Crump ML. 1984. Ontogenetic changes in vulnerability to predation in tadpoles of Hyla pseudopuma. Herpetologica 40:265–271.

Degitz SJ, Holcombe GW, Kosian PA, Tietge JE, Durhan E J, Ankley GT. 2003. Comparing the effects of stage and duration of retinoic acid exposure on amphibian limb development: chronic exposure results in mortality, not limb malformations. Toxicol Sci 74: 139–146.

Eaton BR, Eaves S, Stevens C, Puchniak A, Paszokowski CA. 2004. Deformity level in wild populations of the wood frog (*Rana sylvatica*) in three ecoregions of Western Canada. J Herpatol 38:283–287.

Formanowicz Jr DR. 1984. Foraging tactics of an aquatic insect: partial consumption of prey. Anim Behav 32:774–781.

Forsyth JW. 1946. The histology of anuran limb regeneration. J Morphol 79:287–321.

French V, Bryant PJ, Bryant SV. 1976. Pattern regulation in epimorphic fields. Science 193:969–981.

Fry AE. 1966. Hind limb regeneration in *Rana pipiens* larvae. Copeia 1966:530–534.

Gillilland MG, Muzzall PM. 2002. Amphibians, trematodes, and deformities: an overview from southern Michigan. Comp Parasitol 69:81–85.

Gray RH. 2000. Historical occurrence of malformations in the cricket frog, *Acris crepitans*, in Illinois. Trans Illinois State Acad Sci 93:279–284.

Hairston NG. 1989. Ecological experiments. Cambridge: Cambridge University Press.

Heyer WR, McDiarmid RW, Weigmann DL. 1975. Tadpoles, predation, and pond habitats in the tropics. Biotropica 7:100–111.

Hoppe DM. 2000. History of Minnesota frog abnormalities: do recent findings represent a new phenomenon? In: Kaiser H, Casper GS, Bernstein N, editors. Investigating amphibian declines: proceedings of the 1998 Midwest declining amphibians conference, Volume 107. Cedar Falls, IA: Iowa Academy of Science. p 86–89.

Hoppe DM. 2005. Malformed frogs in Minnesota: history and interspecific differences. In: Lannoo MJ, editor. Amphibian declines: the conservation status of United States species. Berkeley, CA: University of California Press. p 103–108.

Johnson PTJ, Lunde KB, Ritchie EG, Launer AE. 1999. The effect of trematode infection on amphibian limb development and survivorship. Science 284:802–804.

Johnson PTJ, Lunde KB, Haight RW, Bowerman J, Blaustein AR. 2001. *Ribeiroia ondatrae* (Trematoda: Digenea) infection induces severe limb malformations in western toads (*Bufo boreas*). Can J Zool 79:370–379.

Johnson PTJ, Sutherland DR, Kinsella JM, Lunde KB. 2004. Review of the trematode genus Ribeiroia (Psilostomidae): ecology, life history, and pathogenesis with special emphasis on the amphibian malformation problem. Adv Parasitol 57:191–253.

Lannoo MJ, editor. 1998. Status and conservation of Midwestern amphibians. Iowa City, IA: University of Iowa Press. 528p.

Lannoo MJ. 2008. Malformed frogs: the collapse of aquatic ecosystems. Berkeley and Los Angeles, California and London, England: University of California Press.

Lannoo MJ, Sutherland DR, Jones P, Rosenberry D, Klaver RW, Hoppe DM, Johnson PTJ, Lunde KB, Facemire C, Kapfer JM. 2003. Multiple causes for the malformed frog phenomenon. In: Linder G, editor. Multiple stressor effects in relation to declining amphibian populations. Report no. 1443. West Conshohocken, PA: American Society for Testing and Materials. p 233–262.

Levey R, Shambaugh N, Fort, Andrews J. 2003. Investigations into the causes of amphibian malformations in the Lake Champlain Basin of New England. Waterbury, VT: Department of Environmental Conservation.

Manteifel YB, Reshetnikov AN. 2002. Avoidance of noxious tadpole prey by fish and invertebrate predators: adaptivity of a chemical defense may depend on predator feeding habits. Arch Hydrobiol 153:657–668.

McCallum ML, Trauth SE. 2003. A 43-year museum study of Northern cricket frog (*Acris crepitans*) abnormalities in Arkansas: upward trends and distributions. J Wildl Dis 39:522–528.

Meteyer CA, Loeffler KI, Fallon JF, Converse KA, Green E, Helgen JC, Kersten S, Levey R, Eaton-Poole L, Burkhart JG. 2000. Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies. Teratology 62:151–171.

Muneoka K, Holler-Dinsmore G, Bryant SV. 1986. Intrinsic control of regenerative loss in *Xenopus laevis* limbs. J Exp Zool (Mol Biol Evol) 240:47–54.

Ouellet M, Bonin J, Rodrigue J, DesGranges J, Lair S. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free living anurans from agricultural habitats. J Wildl Dis 33:95–104.

Peckarsky BL. 1982. Aquatic insect predator-prey relations. BioSci 32:261–266.

Reeves MK, Dolph CL, Zimmer H, Tjeerdema RS, Trust KA. 2008. Road proximity increases risk of skeletal abnormalities in wood frogs from National Wildlife Refuges in Alaska. Environ Health Perspect 116:1009–1014.

Rohr JR, Schotthoefer AM, Raffel TR, Carrick HJ, Halstead N, Hoverman JT, Johnson CM, Johnson LB, Lieske C, Piwoni MD, Schoff PK, Beasley VR. 2008. Agrochmeicals increase trematode infections in a declining amphibian species. Nature 455:1235–1239.

Scadding SR. 1981. Limb regeneration in adult amphibia. Can J Zool 59:34–46.

Schoff PK, Johnson CM, Schotthoefer AM, Murphy JE, Lieske C, Cole RA, Johnson LB, Beasley VR. 2003. Prevalence of skeletal and eye malformations in frogs from north-central United States: estimations based on collections from randomly selected sites. J Wildl Dis 39:510–521.

Schotté OE, Harland M. 1943. Amputation level and regeneration in limbs of late *Rana clamitans* tadpoles. J Morphol 73:329–363.

Schotthoefer AM, Koehler AV, Meteyer CU, Cole R. 2003. Influence of *Ribeiroia ondatrae* (Trematoda: Digenea) infection on limb development and survival of northern leopard frogs (*Rana pipiens*): effects of host stage and parasite-exposure level. Can J Zool 81:1144–1153.

Sessions SK. 1997. Evidence that deformed frogs are caused by natural phenomena. Special Symposium, 18th annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC), San Francisco.

Sessions SK. 2003. What is causing deformed amphibians? In: Semlitch RD, editor. Amphibian conservation. Washington, DC: Smithsonian Press. p 168–186.

Sessions SK, Ruth SB. 1990. Explanation for naturally occurring supernumerary limbs in amphibians. J Exp Zool 254:38–47.

Sessions SK, Franssen RA, Horner VL. 1999. Morphological clues from multilegged frogs: are retinoids to blame? Science 284:800–802.

Sih A. 1980. Optimal Foraging: partial consumption of prey. Am Nat 116:281–290.

Skelly DK. 2002. Experimental venue and estimation of interaction strength. Ecology 83:2097–2101.

Skelly DK, Werner EE. 1990. Behavioral and life historical responses of larval American toads to an odonate predator. Ecology 71:2312–2322.

Skelly DK, Bolden SR, Freidenburg LK, Freidenfelds NA, Levey R. 2007. *Ribeiroia* infection is not responsible for Vermont amphibian deformities. Ecohealth 4:156–163.

Souder W. 2000. A plague of frogs: the horrifying true story. Minneapolis, MN: University of Minnesota Press.

Stopper G, Hecker L, Franssen RA, Sessions SK. 2002. How parasites cause deformities in amphibians. J Exp Zool (Mol Dev Evol) 294:252–263.

Taylor B, Skelly D, Demarchis LK, Slade MD, Galusha D, Rabinowitz PM. 2005. Proximity to pollution sources and risk of amphibian limb malformation. Environ Health Perspect 113:1497–1501.

Vandenlangenberg SM, Canfield JT, Magner JA. 2003. A regional survey of malformed frogs in Minnesota (USA). Environ Monit Assess 82:45–61.

Veith M, Viertel B. 1993. Veränderungen an den Extremitäten von Larven und Jungtieren der Erdkröte (*Bufo bufo*): analyse möglicher Ursachen. Salamandra 29:184–199.

Wassersug RJ. 1973. Aspects of social behavior in anuran larvae. In: Vial JL, editor. Evolutionary biology of the anurans. Columbia: University of Missouri Press. p 273–298.