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LETTER

An examination of amphibian sensitivity to environmental contaminants: are amphibians poor canaries?

Jacob L. Kerby,[1]* Kathryn L. Richards-Hrdlicka,[2] Andrew Storfer[3] and David K. Skelly[2,4]

[1]*Department of Biology, University of South Dakota, Vermillion, SD 57069, USA*

[2]*School of Forestry and Environmental Studies, Yale University, New Haven, CT 06511, USA*

[3]*Biological Sciences, Washington State University, Pullman, WA 99164, USA*

[4]*Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06511, USA*

**Correspondence: E-mail: jacob.kerby@usd.edu*

Abstract

Nearly two decades ago, the global biodiversity crisis was catapulted to the front pages of newspapers with the recognition of worldwide amphibian declines. Amphibians earned their appellation, 'canaries in a coal mine', because of apparent high sensitivity to human-mediated environmental change. The most frequently cited causes for high susceptibility include permeable skin, a dual aquatic-terrestrial life cycle and a relatively rudimentary immune system. While some researchers have questioned the basis for the canary assertion, there has been no systematic evaluation of amphibian sensitivity to environmental challenges relative to other taxa. Here, we apply a database representing thousands of toxicity tests to compare the responses of amphibians relative to that of other taxonomic groups. The use of standardized methods combined with large numbers of identical challenges enables a particularly powerful test of relative effect size. Overall, we found that amphibians only exhibit moderate relative responses to water-borne toxins. Our findings imply that, as far as chemical contaminants are concerned, amphibians are not particularly sensitive and might more aptly be described as 'miners in a coal mine'. To the extent that amphibian declines have been mediated by chemical contaminants, our findings suggest that population losses and extinctions may have already occurred in a variety of taxa much more sensitive than amphibians.

Keywords

Amphibians, canary in a coal mine, contaminants, declines, indicator species, sensitive species.

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INTRODUCTION

On 22 February 1990 the *New York Times* ran an editorial entitled 'Frogs as Canaries' (1990) that enlisted the use of amphibian species as the proverbial 'canary in a coal mine'. This moniker arose from concerns that widespread declines and extinctions of amphibians may be early indicators of broader environmental degradation that would eventually affect other species and possibly even humans. While it had appeared sporadically before this time, subsequent use of the canary metaphor for amphibians within the media as well as the scientific literature became commonplace (e.g. Cowen 1990; Vitt *et al.* 1990; Morell 1999; Halliday 2000; Norris 2007). Many of these studies presume amphibians to be particularly, and possibly uniquely, sensitive to environmental perturbation. In the wake of evidence that a higher proportion of amphibians are threatened with extinction than any other vertebrate class, scientists and others have attempted to explain the relative fragility of amphibians (Stuart *et al.* 2004). Purported explanations for their apparent sensitivity include permeable skin, a life cycle that most often includes aquatic and terrestrial stages, and a rudimentary immune system (Wake & Vredenburg 2008). However, as several studies have noted, claims for the relative sensitivity of amphibians and those regarding the traits that underlie that sensitivity, have been founded more on argument than on evaluation (Pechmann & Wilbur 1994; Halliday 2000; Beebee & Griffiths 2005). The few studies which have estimated the relative vulnerability of amphibians to environmental challenges have not yielded a consistent pattern with some suggesting a large effect (Sparling *et al.* 2001; Sparling 2003; Relyea *et al.* 2005) and others suggesting that amphibians are not particularly sensitive (Thurston *et al.* 1985; Hall & Henry 1992; DeYoung *et al.* 1996; McCrary & Heagler 1997). Reviewing this body of evidence, Collins & Crump (2009) in their recent book on amphibian declines have argued that amphibians should not be held up as an early warning system for ecosystems.

The claims, counterclaims and a relative paucity of data provide the impetus for the present study. We had as our goal a more inclusive and comprehensive evaluation of responses to comparable challenges across a set of taxa that included amphibians. We focused on chemical contaminants for two reasons. First, the potential role of chemicals in amphibian declines has been implicated repeatedly during the last two decades of amphibian research (Blaustein *et al.* 2003; Collins & Storfer 2003; Collins & Crump 2009). Their role in species declines and disappearances remains unclear in many cases, yet there are a number of lines of evidence suggesting such a role for chemical exposure (Davidson *et al.* 2002; Davidson 2004; Gibbs *et al.* 2009). Second, there is a wealth of data examining their potential for effects on many taxa. In addition, there is increasing evidence that contaminants reduce amphibian fitness directly (Hayes *et al.* 2002; Sparling 2003). They also may interact to cause significant increases in disease susceptibility (Forson & Storfer 2006; Johnson *et al.* 2007; Rohr *et al.* 2008) and reduce survival when combined with other stressors (Relyea & Mills 2001; Bancroft *et al.* 2008).

Contaminants offer a number of advantages as an index of relative sensitivity. First, impacts of different contaminant types can occur via a variety of physiological pathways. Second, there are many thousands of contaminants continually released into the environment making them a practically omnipresent feature, even in pristine areas (e.g. McConnell *et al.* 1998). Finally, in the context of product development and required regulatory review testing, an enormous number of toxicological studies have been conducted, exposing hundreds of different species to thousands of different chemical agents. Tests have been carried out under controlled laboratory conditions using standardized protocols designed specifically to facilitate comparative analyses. Amphibians have figured prominently in recent toxicological testing, thereby facilitating direct comparisons of sensitivity between amphibians and other taxa.

As a result, we aim to compare the average lethal impacts of several contaminant types on amphibians to the lethal impacts on all available taxonomic groups. While there will be clear variation in tolerance by species and by chemicals, the goal of this analysis is to test the claim that amphibians can serve as an important taxon for signalling impacts to water quality, or as a key sensitive group for the wider biodiversity crisis in general.

METHODS

Using the United States Environmental Protection Agency's (US EPA) Aquatic Toxicity Information Retrieval (AQUIRE) database, we collected and analysed results from lethality tests from more than 28 000 studies including 1279 species and 107 chemical agents. These data were used to build species sensitivity distributions (SSDs) (Posthuma *et al.* 2002) for each of 13 major taxonomic groups (Actinopterygii, Amphibia, Arachnida, Bivalvia, Branchiopoda, Gastropoda, Insecta, Malacostraca, Maxillopoda, Monogononta, Oligochaeta, Polychaeta and Secernentea) examining responses to four major contaminant types (pesticides, heavy metals, inorganics and phenols).

Data used for these analyses were obtained from the 7 September 2007 update of the U.S. Environmental Protection Agency's AQUIRE database (http://www.epa.gov). We focused on aquatic egg and larval stages of amphibians; the number of adult amphibian studies was inadequate for rigorous analyses. Acute lethal toxicity studies (24–96 h) provided the best opportunities for comparative analyses. These toxicity studies are a staple of environmental toxicology and employ a dose–response curve model to estimate concentrations of contaminants where 50% of the individuals exposed are killed (LC_{50}). This 50% mark is employed as a standard primarily because it is the most reliable point to estimate on the curve and therefore provides the best measure for comparisons between both contaminants and species.

To compare other taxa with amphibians specifically, we initially extracted all studies on chemicals that had been tested on amphibians. From this subset, we classified species to the taxonomic level of class and assigned chemicals into four major types of contaminants: heavy metals, inorganics, phenols and pesticides. From these data, we created SSDs to examine the relative impacts of each group on several taxa. Just as dose–response curves for individual species provide an estimate of toxicity amongst a variation of responses, SSDs employ this same methodology by using species values as individual data points. In order to make reliable estimates, we followed US EPA protocol for construction of SSDs, and required a minimum of seven different species within each taxon to warrant inclusion in the analysis. Each data point represents an average LC_{50} value for a species within the taxon (a geometric mean was used where multiple LC_{50} values existed for a particular species–chemical combination). Just as toxicity tests estimate effect size via LC_{50}, SSDs provide analogous estimates of hazardous concentrations (HC_{50}). Best fit lines were run through the data points to estimate HC_{50}values, the estimates of where 50% of species within a taxon exhibit at least 50% mortality (Posthuma *et al.* 2002). General linear models were used to generate and compare best fit lines between taxa for each chemical type.

HC_{50} estimates were used from these models and examined via a Dunnet comparison of amphibians with all other taxa. Due to the large amount of available data on pesticides, this analysis was able to be subsequently run on four major pesticide classes: pyrethroids, carbamates, organophosphates and organochlorines.

RESULTS

Our final analysis incorporated data from 23 942 acute toxicity evaluations conducted across 1075 different invertebrate and vertebrate species challenged by a total of 73 different chemical substances (Table 1). The 44 amphibian species included in the analysis are both taxonomically diverse and widely distributed geographically (Table 2).

For three of four contaminant types, amphibians display relatively low to moderate sensitivity to chemical exposure (pesticides, heavy metals and inorganics; Fig. 1). In all three cases, the estimated HC_{50} values were above the average estimates for all taxa analysed (Table 3) signifying an overall low relative sensitivity. Branchiopods instead were the most sensitive taxon to metal and inorganic exposures, while not surprisingly insects were the most sensitive to pesticide exposure. Amphibians were highly sensitive to the final contaminant type, phenols. Only one (Monogonota) of the other 11 taxa registered a lower HC_{50} value, and this value was not statistically significant from the amphibian measure (Table 3). When the four pesticide types (pyrethroids, carbamates, organophosphorus and organochlorines) were analysed individually, we again found similar low to moderate amphibian susceptibilities as determined in the analysis of all pesticides combined (Fig. 2).

DISCUSSION

Amphibians as canaries?

This first systematic, broad scale analysis provides little evidence that amphibians are the most sensitive taxon to chemical contaminants, an important and ubiquitous source of environmental perturbation. These findings offer critical context to earlier studies providing direct experimental comparisons of small numbers of species and chemical agents (Thurston *et al.* 1985; Hall & Henry 1992; DeYoung *et al.* 1996; McCrary & Heagler 1997) which revealed a wide range of effects – from none to severe – without providing a sense of overall sensitivity.

While most attention in the literature focuses on amphibian sensitivity to heavy metal and pesticide exposure (see review in Sparling 2003), there is no evidence of heightened sensitivity in either case. After bivalves and insects, amphibians are the least sensitive to inorganic chemical exposure. By contrast, few studies focus on amphibian responses to phenols; however, it is to this group of chemical agents that amphibians display a very high relative sensitivity. In comparison with the number of studies that have been conducted on other contaminant groups, phenol impacts have been largely overlooked. This particular contaminant group deserves much further scrutiny because it could play a key role in impacting amphibian populations. Of particular interest is Triclosan, which is widely used as an antibacterial agent in several household cleaners and personal care products. While most of the attention on this chemical has been on its role in selecting for resistance among bacterial strains, one study has found it to disrupt endocrine function in bullfrogs (Veldhoen *et al.* 2006). If amphibians were to serve the role as a canary in a coal mine for a particular group of chemicals, phenols seem to be the most likely candidate. It must be noted, however, that in the database, this group consisted of only three chemicals and the relative sensitivity could shift significantly with the inclusion of more studies from other phenolic compounds.

The actual canaries that were used in coal mines were not used to detect all forms of dangerous contaminants, so it seems a bit simplistic to apply this concept to amphibians as well. Canaries were used to detect increased levels of methane or carbon monoxide gas only. Miners were likely to encounter a large host of other toxic compounds in their work, particularly metals, for which the canaries served no function in alerting the miners. In a similar vein, the rapid loss of amphibian populations can potentially point us to an area of concern, but it cannot represent a global estimate of human impact. While there are clear examples of amphibians being impacted by pesticide residues in ponds, there are many other examples of amphibian populations persisting in similarly polluted areas (e.g. Rubbo & Kiesecker 2005; D'Amore *et al.* 2009). The persistence of these 'canaries' might send a dangerous message that these areas are without impact, when we can see from the data here that it is far more imperative to examine the invertebrate fauna instead.

While amphibians, as a group, are not among the most sensitive taxa, our results suggest that particular amphibian

Table 1 Total number of contaminant types examined within each group

Heavy metals	11
Inorganics	17
Phenols	3
Pesticides	42
Pyrethroids	3
Carbamates	5
Organophosphates	9
Organochlorines	13
Grand total	**73**

Table 2 List of amphibian species examined in each contaminant group

	Inorganics	Pesticides	Heavy metals	Phenols
Anurans	*Bufo americanus* (5) *Bufo americanus americanus* (2) *Bufo arenarum* (4) *Bufo bufo* (1) *Bufo melanostictus* (6) *Microhyla ornata* (8) *Pseudacris triseriata triseria* (1) *Rana catesbeiana* (3) *Rana clamitans melanota* (1) *Rana cyanophlyctis* (4) *Rana hexadactyla* (16) *Rana pipiens* (1) *Xenopus laevis* (3)	*Acris crepitans* (2) *Adelotus brevis* (9) *Bufo americanus* (12) *Bufo arenarum* (6) *Bufo bufo japonicus* (75) *Bufo marinus* (6) *Bufo melanostictus* (7) *Bufo vulgaris formosus* (7) *Bufo woodhousei* (1) *Bufo woodhousei fowleri* (66) *Hyla versicolor* (1) *Limnodynastes peroni* (9) *Microhyla ornata* (8) *Pseudacris triseriata* (23) *Pseudacris triseriata triseria* (27) *Rana brevipoda porosa* (5) *Rana catesbeiana* (9) *Rana chensinensis* (2) *Rana clamitans* (18) *Rana hexadactyla* (30) *Rana limnocharis* (18) *Rana pipiens* (13) *Rana ridibunda* (1) *Rana sphenocephala* (16) *Rana sylvatica* (2) *Rana temporaria* (22) *Rana tigrina* (10) *Scinax nasica* (4) *Xenopus laevis* (40)	*Adelotus brevis* (3) *Bufo bufo japonicus* (1) *Bufo marinus* (3) *Bufo melanostictus* (12) *Bufo woodhousei fowleri* (1) *Gastrophryne carolinensis* (1) *Hyla chrysoscelis* (1) *Limnodynastes peroni* (3) *Microhyla ornata* (18) *Rana breviceps* (3) *Rana cyanophlyctis* (4) *Rana heckscheri* (1) *Rana hexadactyla* (16) *Rana pipiens* (1) *Rana sphenocephala* (1) *Rana temporaria* (3) *Rana tigrina* (11) *Xenopus laevis* (6)	*Bufo boreas* (1) *Bufo bufo japonicus* (7) *Rana catesbeiana* (2) *Rana hexadactyla* (4) *Rana limnocharis* (1) *Rana sphenocephala* (1) *Xenopus laevis* (19)
Caudates	*Ambystoma maculatum* (18) *Ambystoma opacum* (6)	*Ambystoma maculatum* (2) *Ambystoma opacum* (2) *Hynobius retardatus* (2) *Triturus cristatus* (3)	*Ambystoma mexicanum* (3) *Ambystoma opacum* (1) *Eurycea bislineata* (1)	*Ambystoma mexicanum* (12)

Number in parentheses represents the number of studies included per species.

species are highly sensitive to particular chemicals or chemical groups relative to other organisms. That is to say that just because on average amphibians are not the most sensitive taxon, this does not mean that highly vulnerable amphibian species are not being impacted. There have been numerous studies indicating the sensitive nature of amphibian exposure to contaminants (see reviews by Sparling 2003; Relyea *et al.* 2005). The US EPA database of toxicity tests can also serve to warn of further impacts. As one example, the Tiger Frog (*Rana tigrina*) is strongly sensitive to the organophosphate endosulfan. This species is distributed throughout India where exposure to this type of chemical is likely and for which it may be able to serve as a pertinent early indicator of contamination. Further analyses will undoubtedly uncover a number of these confluences between the degree of sensitivity in laboratory tests and the likelihood of exposure suggesting that the canary metaphor may continue to be useful for particular amphibian species, and indeed other taxa, when based on supporting data on particular contaminants. However, the current approach to regulatory testing in the United States relies heavily on a single species, the African Clawed Frog (*Xenopus laevis*) not native to North America and, in our analyses, not particularly sensitive to any type of chemical. Despite being more cumbersome to implement, a more customized regional approach (driven by relevant local species) might more reliably estimate contaminant exposure and impacts on differing amphibian species.

Basing our analyses upon LC_{50} values from acute toxicity tests offered a number of critical methodological advantages

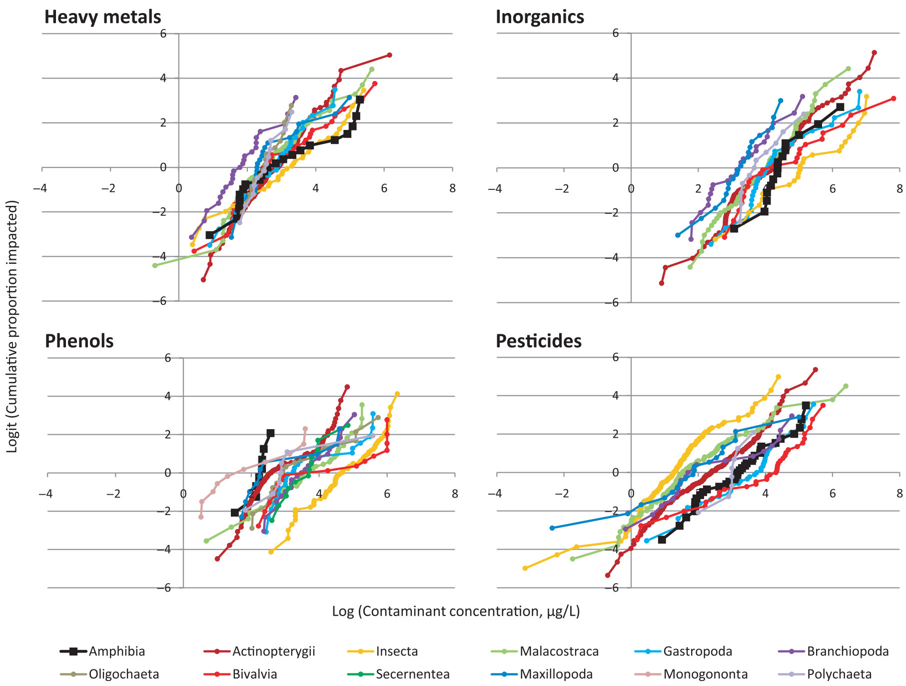

Figure 1 Species sensitivity distributions (SSDs) for four contaminant types. Each point represents an average LC_{50} value for a single species grouped into one of 12 taxonomic groups. The *y*-axis represents the cumulative proportion of species within a taxon that are vulnerable at a particular concentration. The intersection of each line with the *x*-axis represents the log concentration at which half of the species within a taxon are estimated to be vulnerable.

including the enormous number of comparable tests completed as well as a relevant end point. Nevertheless, there is good evidence that some adverse effects from exposure may not manifest during the short duration of an acute evaluation (Rohr *et al.* 2006b; Relyea & Diecks 2008; Jones *et al.* 2009). In this sense, responses to acute exposure highlighted here are just one variety of effect. Our focus on acute responses should not be taken as a comprehensive picture of adverse influences from chemical exposure any more than studies of chronic exposure to low contaminant

Table 3 Estimated HC_{50} values for each taxon for the four contaminant types

	Phenols		Metals		Pesticides		Inorganics	
Taxon	HC_{50}	SE	HC_{50}	SE	HC_{50}	SE	HC_{50}	SE
Actinopterygii	2.88*	0.10	2.84	0.07	2.37*	0.08	4.08*	0.08
Malacostraca	3.61*	0.19	2.59*	0.12	1.78*	0.14	3.96*	0.1
Gastropoda	2.62*	0.23	2.70	0.15	3.42	0.2	4.3	0.19
Oligochaeta	3.32*	0.26	2.58	0.12	–	–	–	–
Branchiopoda	3.57*	0.17	1.81*	0.16	2.3*	0.34	3.2*	0.19
Insecta	4.73*	0.12	3.14	0.23	1.25*	0.09	5.13*	0.26
Bivalvia	4.09*	0.39	2.80	0.17	3.71*	0.25	4.49	0.28
Maxillopoda	2.76	0.36	2.56	0.18	1.76*	0.36	3.21*	0.16
Polychaeta	3.1*	0.44	2.49*	0.13	2.97	0.16	3.82*	0.19
Secernentea	3.54*	0.18	–	–	–	–	–	–
Monogononta	1.87	0.39	–	–	–	–	–	–
Amphibia	**2.20**	**0.11**	**3.01**	**0.29**	**3.15**	**0.19**	**4.48**	**0.19**
Average	3.19		2.65		2.52		4.07	

HC_{50} values represent the estimated average toxicity value for each taxon (log μg L^{-1}).
*Significant differences ($P < 0.05$) between HC_{50} values for particular taxon and amphibians.

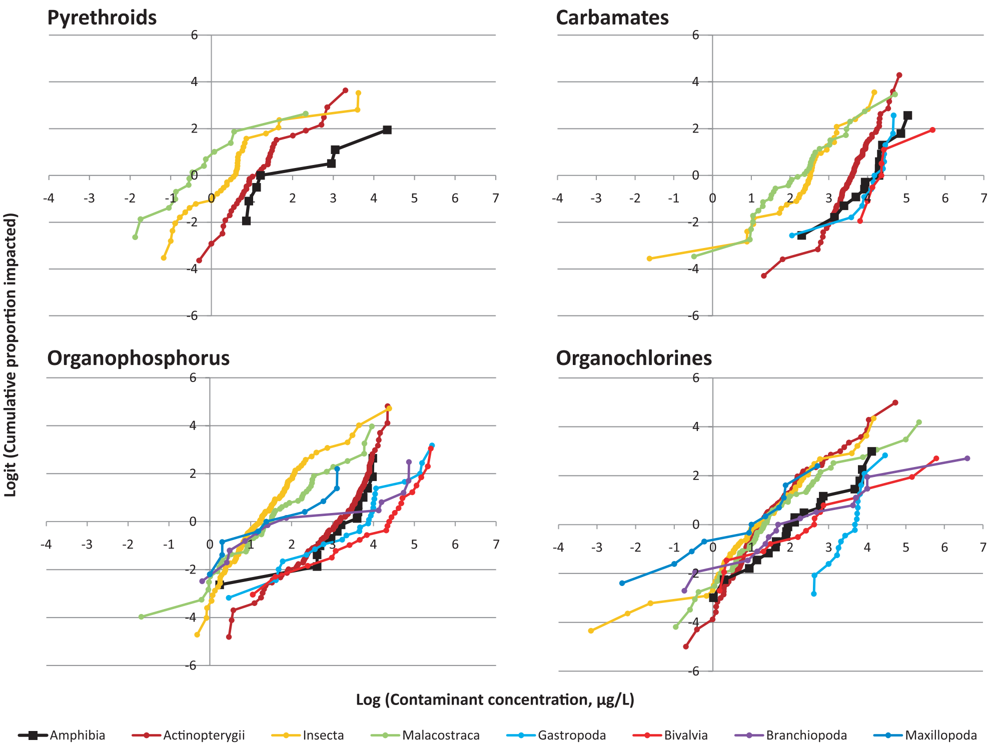

Figure 2 Species sensitivity distributions for the four available pesticide classes. Each point represents an average LC_{50} value for a single species grouped into one of eight taxonomic groups. The *y*-axis represents the cumulative proportion of species within a taxon that are vulnerable at a particular concentration. The intersection of each line with the *x*-axis represents the log concentration at which half of the species within a taxon are estimated to be vulnerable.

concentrations (e.g. Glennemeier & Denver 2001) or those mediated through food web influences (e.g. Boone & Semlitsch 2001) offer a complete picture. It will be critical to evaluate a greater variety of effects as the number and diversity of exposure studies grows (Rohr *et al.* 2006a). Further, it is unclear how acute toxicity studies conducted on amphibian larvae relate to population-level responses (Vonesh and De la Cruz 2002; Schmidt 2004). Nonetheless, studies of acute effects offer the best opportunity, at present, for a comparative and quantitative comparison of influences across taxa.

While our analyses suggest that amphibians as a group are not particularly sensitive to the majority of contaminants, it remains that natural populations appear to be declining, and perhaps in part, because of such exposure. Analyses of responses to other environmental challenges (e.g. infectious disease) could reveal that amphibians are unusually sensitive to other stressors. Alternatively, the impact of multiple stressors or community context could be heightened in amphibians relative to other taxa (Relyea *et al.* 2005; Rohr *et al.* 2006a). Nonetheless, if a canary is meant to provide an early warning of environmental harm, then the widespread decline of amphibians with apparent moderate sensitivity implies that environmental harm may be well under way. If amphibians worldwide are being affected, our results suggest that other more sensitive taxa may be even more gravely affected. Several community ecology-based studies have shown that the primary impacts on amphibians in fact originate from indirect effects (Relyea *et al.* 2005; Relyea & Diecks 2008). That is, contaminants directly impact amphibian resources and therefore only indirectly reduce their fitness. Clearly, impacts on these more sensitive phytoplankton and zooplankton will not only reduce amphibian diversity but also will likely reverberate throughout the food web. Cross taxonomic comparisons of species declines could provide a clearer indication of the degree to which exposure to environmental challenges is occurring and perhaps even some evidence to the nature of the exposure.

Early considerations of amphibian declines dubbed amphibians as symbols of the biodiversity crisis and as potential warning indicators of more trouble to come (Wake 1991). In one sense, these forecasts were accurate. Twenty years later, amphibians are estimated to be going extinct at 200 times the background rate (Roelants *et al.* 2007) as many of the world's ecosystems have continued to deteriorate. It does not necessarily follow, however, that amphibians are the most sensitive to environmental perturbation. It is also possible, and indeed likely, that other taxa have been undergoing comparable but less-studied declines and extinctions. As a group, the response of amphibians to chemical agents suggests that they are closer to symbolically representing 'miners in a coal mine' and that the harm done to other taxa may be more severe than is typically assumed.

In addition to amphibians not serving as adequate canaries, a broader corollary is that there is no evidence that any of the major taxonomic categories investigated can consistently serve a canary role (at least with regard to contaminants). Nevertheless, the goal of using organisms to provide indicators of environmental health remains critically important and worthy of further development. Systematic approaches to compare relative sensitivity to other environmental threats combined with tests that match particular species with particular challenges may be germane to success. While some species of amphibians and other taxa remain resilient, there are clear examples of particular species that could serve well as canaries. It is crucial to improve our understanding of indicator species so that environmental harms can be rapidly identified and addressed. The critical question remains, 'Will we be able to determine appropriate canaries before it is too late?'

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