What’s Happening to Our Frogs?

(See Burkhart et al., p. 841)

Since the 1980s, there has been increasing attention in the scientific community to the possibility of a worldwide decline in amphibian populations [for example, Carey and Bryant (7)]. Concerns about amphibians further heightened in 1995 when middle school students reported on the Internet (2) a high incidence of malformed frogs from a southern Minnesota farm pond (3). Indeed, there is now a website devoted to reporting such field observations (4). Concurrent with the growing field observations have been efforts to understand the etiology of the malformations and to understand their importance to both wildlife and human health. Many causes have been suggested for the malformations, including environmental chemicals, ultraviolet (UV) light, and parasites. For example, it was recently reported that some degradation products of the insect growth regulator $S$-methoprene alter early frog embryo development in the laboratory (5), and another group has implicated agricultural contaminants in the hindlimb deformities in frogs from a number of ponds in Quebec (6). However, cause-and-effect relationships in the ambient environment remain obscure. In this issue, Burkhart et al. report results of the first laboratory-based effort using pond water samples to investigate potential causes for the recent observations of malformations in amphibian populations in Minnesota.

Specifically, Burkhart et al. employed the Frog Embryo Teratogenesis Assay-Xenopus (FETAX), in which Xenopus laevis (the laboratory rat of amphibian toxicology, and a commonly used model in developmental biology) embryos were exposed beginning with stage 8 (the blastula) and continuing for 96 hr, at which time controls are generally at stage 46 (the time of first appearance of the hindlimb buds). The viability and morphology of larvae were then assessed. They found that growing Xenopus embryos in water sampled from three ponds with reported increased incidences of malformations in native anurans induced high frequencies of abnormalities and death, whereas water from ponds with no reported effects did not.

What do Burkhart et al.’s data tell us? Clearly, something is different between the reference sites and the affected ones that FETAX is able to detect. Does the fact that Xenopus is not a native North American species diminish the relevance to the impacted indigenous species? Probably not, as development is highly conserved across species and even phyla. What about the issue that standard FETAX results cannot recapitulate the limb malformations observed in wild populations of adult frogs because the observation period terminates prior to emergence of the limbs? This presents some potential problems in interpretation, especially because the affected pond waters also tended to be highly lethal to the Xenopus larva in the laboratory, i.e., many of the animals would not have survived to develop limbs. Still, there was a clear biologically adverse response. Does the fact that reference sites were selected by county proximity “without consideration of land use, topology, or limnology” indicate that more may be different about the sites than just the presence of impacted anurans? Perhaps. And does the lack of identification of the specific sites (done to protect the landowners), which precludes independent confirmation of the findings by other scientists (if time has not already changed the conditions of the ponds), diminish the significance of the findings? Perhaps not, but something should be done to rectify this departure from standard scientific procedure. Finally, we need to ask whether the findings have predictive significance for other pond samples, other wildlife species, and in the larger context, for humans. Is the concern for humans heightened by the report of increased rates of birth anomalies in regions of Minnesota associated with pesticide use (7)? Many would probably agree with this, but others would wait until we understand what the assay is actually telling us.

The FETAX assay has been used for several purposes, including the traditional hazard identification of individual chemicals and some mixtures (8,9), as well as environmental monitoring of areas of relatively high contamination (10,11). However, the application to assessment of possible trace level contamination in environmental samples is novel. Is an assay that has historically been used for hazard identification purposes up to this task of determining cause-and-effect relationships for events in the natural environment? There are pronounced differences between the solution used for the control groups and the ionic composition of natural pond water (at least as far as the sampled ponds go). For example, the FETAX control solution contained 272 ppm Na+, 15.6 ppm K+, 19.6 ppm Ca²⁺, and 14.88 ppm Mg²⁺. In contrast, the concentration ranges in the pond water samples (in ppb) were 469–2,876 for Na⁺, 1,544–3,776 for K⁺, 4,759–42,339 for Ca²⁺, and 1,107–20,608 for Mg²⁺. Is something missing from the water, or was some novel toxicant present? In their support, the experiments by Burkhart et al. indicate that there is not a lack of nutrients. For example, simulating the concentrations of Ca, Mg, Na, and K present in the pond waters suggest that low ion concentrations were not a contributing factor (although diluting the pond water with as little as 25% control FETAX solution essentially eliminates the response, and diluting with reference site water yields a more graded concentration–response relationship). In addition, boiling or filtering the water to remove microorganisms did not modify the response, whereas using other extraction techniques to remove inorganic ions (mixed bed ion exchange), small polar molecules (activated charcoal), and uncharged lipophilic substances (a C-18 column) did. Combined,

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these data suggest that no simple ionic difference is responsible and that it is the presence of some active agent, rather than the lack of one, that drives the FETAX response. Nevertheless, the possibility still exists that embryos developing in a less than optimal situation are predisposed to insult by some trace contaminant. In this regard, Luo et al. (12) have shown that magnesium supplementation or deprivation can influence the toxicity of several other divergent cations including nickel, cobalt, zinc, and cadmium. Further complicating the matter, Tietge et al. (13) presented observations of density-dependent effects using the same assay and pond water from an affected site in Minnesota at the 1998 SETAC meeting (14,15). The results suggest that some trace nutrient may be lacking in the affected pond waters, or that the concentration procedures provide an environment that ameliorates a unidirectional action of some trace contaminant? Are these the same sites, and why the apparent differences between the laboratories? What other factors or conditions might be responsible for the amphibian malformations? Research presented at the 1998 SETAC meeting (14,15) provides additional information on potential causes. Ankley and co-workers (15) tested the effects of methoprene and UV light, alone and in combination, on development of the northern leopard frog, one of the impacted natural species. They found that, regardless of the presence of methoprene, exposure to UV light induced a high incidence of hindlimb malformations (this study was of sufficient duration to actually observe the morphology of the limbs). So now we have studies that show that methoprene degradation products and UV light are both detrimental to amphibian development. But the full range of malformations seen in native populations has not been reproduced in any of the laboratory studies. And what about combinations of stressors? Like much research, especially at the early discovery phase, the efforts to elucidate the causal factors for the apparently recent and generally widespread appearance of malformations in native frogs raise more questions than they answer. It is admittedly easy to demonstrate the limitations of any foray into new research areas or those that challenge our usual scientific assumptions. The concerns raised above do not diminish the potential importance of the findings of Burkhart et al. because the response in FETAX in this situation may be telling us something very important. We must be smart enough to figure what that is and quickly move to determine if a particular environmental contaminant(s) is responsible. If so, we must determine what can be done about it. Is it something new to the environment, or have we just not been observant enough to know that it has been exerting effects for some period of time? Perhaps it is not an anthropogenic contaminant at all. Andersen (16) recently reported that an as-yet-unnamed fungus appears to be responsible for the decline of several frog species around the world. The fungus appears to impact the skin, resulting in the laying down of extra layers of keratin, thus impacting transdermal respiration. Is this involved in the Minnesota situation? If it is, has the impact been heightened by presence of anthropogenic stressors? The questions just continue to build.

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