

*The Global Environment***Identifying the Environmental Culprit Harming Amphibians**

What started out as a relatively standard biology field trip in Minnesota in 1995 to collect frogs turned into a bizarre experience. Approximately half of the frogs students collected were deformed, with extra legs or missing legs or no eyes. Turning to the Internet, the class soon discovered that the problem was not isolated to Minnesota. Neighboring states were reporting the same phenomenon—an alarming number of deformed frogs, all across the United States and Canada.

Some environmental scientists suspected that chemical pollutants in the water might be causing the deformities and that the widespread occurrence of deformed frogs might well be an early warning of potential future problems in other species, including humans.

Other scientists cautioned that a different factor might be responsible. Although chemicals such as pesticides certainly *could* produce deformities in localized situations, say near a chemical spill, so too could other environmental factors affecting local habitats, such as parasitic infections. Demonstrating this point, researchers in 1999 showed that the multilimb and missing limb phenomenon in frogs can be caused by parasites that infect the developing tadpoles, disrupting development of their limbs.

Responding to this alternative suggestion, those scientists nominating pesticides as the principal culprit have cautioned that showing parasites can have a significant effect on local populations is not the same thing as demonstrating that they have in fact done so. And, they add, it certainly doesn't rule out a major contribution to the problem by environmental pollutants.

Although deformed frogs quickly received national attention, they are but the tip of the iceberg, a global problem of declining amphibian populations. During the past 50 years, there has been a worldwide catastrophic decline in amphibian populations. In some cases we can point to specific local human activities as the cause: habitat destruction, the introduction of competitive species, and industrial pollution.



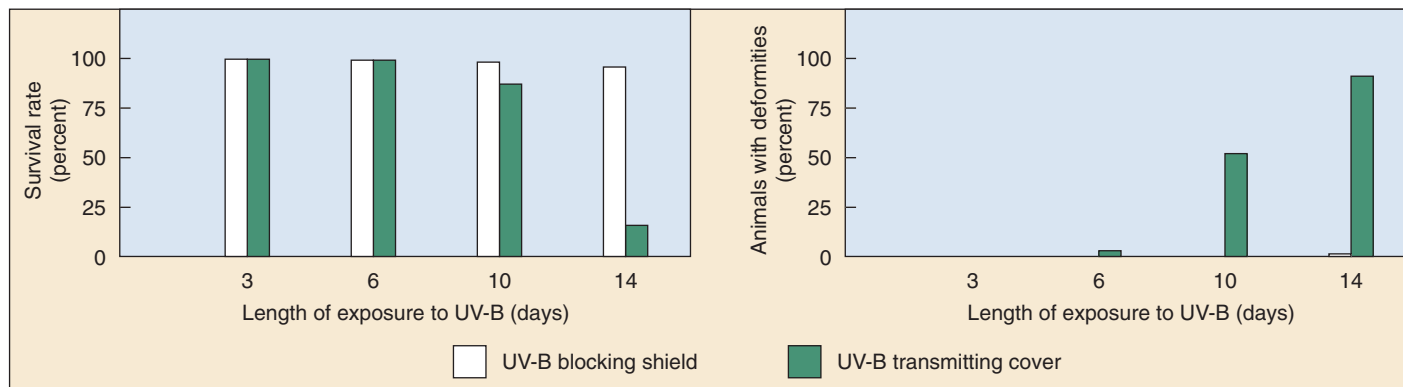
Disappearing amphibians. Populations of amphibians, like this Western toad (*Bufo boreas*) are declining in numbers in many regions.

However, because the problem appears to be global in nature, we must also consider the possibility that the decline of amphibians reflects some more global environmental change. Chemical contamination of water by acid rain, increasing ionizing radiation (UV-B) resulting from ozone layer depletion, changes in weather patterns caused by the warming of the atmosphere—all have been seriously proposed as potential global causes of declining amphibian populations.

Amphibians are not the only species experiencing declining populations. Songbirds in the eastern United States, for example, have declined precipitously in the last few decades, largely due to habitat destruction. So why focus on amphibians? While amphibians play a significant role in the ecological balance of many habitats, and have proven effective in the development of new drugs, their real importance to biologists is that amphibians are particularly sensitive indicators of the environment. Their semi-aquatic mode of living, depending on a watery environment to reproduce and keep their skin moist, means that they are exposed to all types of environmental changes.

Amphibians are particularly vulnerable during early development, when their fertilized eggs lay in water, exposed to acid, chemical pollutants, and UV-B radiation. While numerous experiments performed under laboratory conditions have demonstrated the power of these factors to produce developmental deformities, and in so doing to reduce population survival rates, it is important to understand that “can” does not equal “does.” To learn what is in fact going on, it is necessary to also examine the effects of these factors on amphibian development in the natural environment.

In a particularly clear example of the sort of investigation that will be needed to sort out this complex issue, Andrew Blaustein of Oregon State University headed a team of scientists that set out to examine the effects of UV-B radiation on amphibians in natural populations. In a series of experiments carried out in the field, they attempted to assess the degree to which UV-B radiation promoted amphibian developmental deformities under natural conditions.



Blaustein's UV-B experiment. In the group of salamanders whose eggs were protected from UV-B radiation, hatching rates were higher and deformity rates were lower.

The Experiment

Laboratory experiments examining the affects of UV-B on amphibian development had already shown a significant increase in embryonic mortality in some amphibian species, and not in others. Why only in some?

Perhaps behaviors shared by many amphibian species might lead to an increased susceptibility to damage from UV-B radiation, behaviors such as laying eggs in open, shallow waters that offer significant exposure to UV-B radiation. Perhaps physiological traits of certain species make them particularly susceptible to damage from UV-B radiation, traits such as low levels of photolyase, an enzyme that removes harmful photoproducts induced by UV light.

Blaustein's group selected specimens for two different experiments that exhibit these factors, the long-toed salamander, *Ambystoma macrodactylum* and several species of frogs.

The goal of Blaustein's field experiment was to allow fertilized eggs to develop in their natural environment with and without a UV-B protective shield. Eggs in both groups were monitored for the appearance of deformities and for survival rates. Eggs were collected from natural shallow water sites (<20 cm deep) and randomly placed within enclosures containing either a UV-B blocking Mylar shield or a UV-B transmitting acetate cover (50 eggs per each enclosure replicated four times). The enclosures were placed in small, unperforated plastic pools containing pond water and the pools were placed back in the pond thereby exposing the eggs and developing embryos to ambient conditions. The UV-B blocking Mylar shield filtered out more than 94% of ambient UV-B radiation, while the UV-B transmitting acetate cover allowed about 90% of ambient UV-B radiation to pass through.

The Results

Embryos under the UV-B shields had significantly higher hatching rates and fewer deformities compared with those under the UV-B transmitting acetate covers (see graphs above). Of the 29 UV-B exposed individuals that

hatched, 25 had deformities. This is significant compared to the 190 UV-B shielded individuals that hatched and only 1 showed deformities. These results support the hypothesis that naturally occurring UV-B radiation can adversely affect development in some amphibians, inducing deformities.

Blaustein's group speculates that the higher mortality rates and deformities in frogs and other amphibian species might in fact be due to lower than normal levels of photolyase activity in their developing eggs and embryos, low levels such as found naturally in salamanders.

Laboratory and field experiments seem to support this idea. For one thing, frog species that are not sensitive to UV-B have very high photolyase activity levels. Evaluating 10 different species, Blaustein's team found a strong correlation between species exhibiting little UV-B radiation effects and higher levels of photolyase activity in developing eggs and embryos.

In these experiments, the Pacific tree frog (*Hyla regilla*)—whose populations have not been declining—exhibited the highest photolyase activity and was not affected by UV-B radiation, showing no significant increases in mortality rates in UV-B exposed individuals.

In parallel experiments, the Cascades frog (*Rana cascadae*) and the Western toad (*Bufo boreas*)—both of whose populations have been experiencing markedly declining numbers—had less than one-third the photolyase activity seen in *Hyla*, and were strongly affected by UV-B radiation, showing significant increases in mortality rates when exposed to UV-B radiation.

These results suggest that increased level of UV-B radiation resulting from ozone depletion may indeed be a major contributor to amphibian decline—in populations with low photolyase activity. Could chemical pollutants be acting to lower activity levels of this key enzyme? The investigation continues. Undoubtedly, many factors are contributing to declining amphibian population, and there are not going to be many simple answers.