Phil Dotson; Photo Researchers, Inc.

# Amphibians in a Bad Light

*What is killing the eggs of Oregon's western toad?*

by Andrew R. Blaustein

Each spring, two or three times a week, my graduate students and I travel eighty miles east, from Oregon State University to Lost Lake in the Cascade Range. Surrounded by snow-capped peaks and volcanic debris, the lake, at an altitude of about 4,000 feet, is the lowest in the area and consequently the first to lose its ice covering. And when the ice goes, western toads (*Bufo boreas*) immediately begin breeding by the hundreds. Because this can happen any time in May or June, frequent visits insure that we catch them in the act. But it is not easy; some years, when the snowpack is great, we have to snowshoe several miles around the lake to get to the breeding site.

This year, however, the snow around the lake melted by early May, so we could drive close to the small section of the lake that these amphibians seem to prefer. Toads began to emerge on May 6, the earliest date we had ever seen them arise from their six-month winter sleep. We donned our waders, picked up our pails, nets, scales, and notebooks, and ventured into the cold, clear water. After some searching, we found hundreds of toads.

To obtain a long-term record of their reproductive patterns and changes in their population size, we had been weighing, measuring, and marking western toads at Lost Lake since 1979. Nevertheless, we were still amazed at how the toads, in their quest to mate, scrambled over snow and ice and into near-freezing water. Close to the surface, where the toads congregated, violent winds and snow, accompanied by

*Before the snow has completely melted in Oregon's Cascade Range, toads, frogs, and salamanders breed in the clear waters of Lost Lake, above. The western toad, right, is in decline throughout most of its range.*
D. Grant Hokit

Edward R. Degginger; Bruce Coleman, Inc.

*In shallow water, a single strand of western toad eggs, below, lies exposed to the sun's ultraviolet rays. Most salamanders, however, lay their eggs in deep water or other protected places. Inside their eggs, right, embryonic salamanders have already developed their feathery external gills.*

Alan Blank; Bruce Coleman, Inc.

pelting hail, continued to buffet them while they bred.

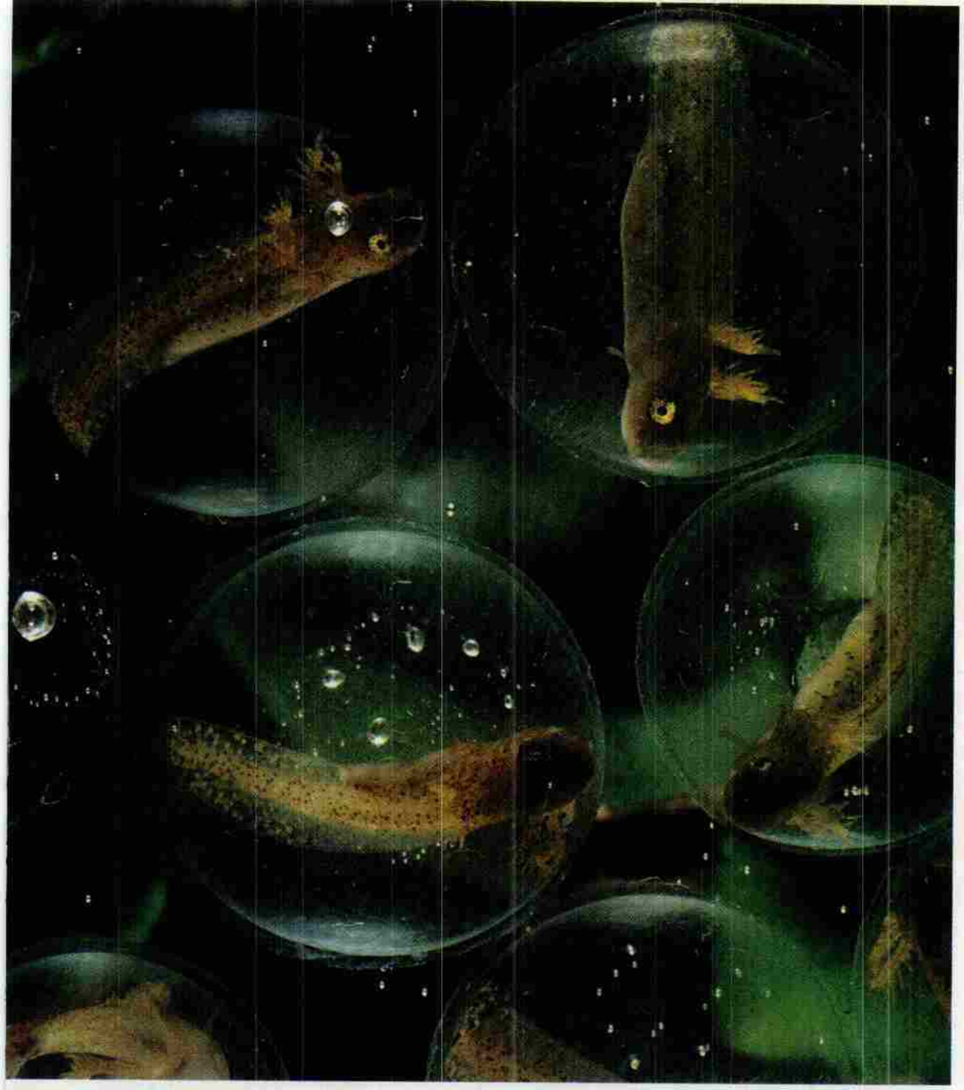
Males arrived first, followed by females, and in a seemingly haphazard manner, hundreds of toads searched for mates. Because the males outnumbered females, the competition for access to females was intense and shoving matches often erupted. Soon after the pairs formed, the females began laying eggs and the males fertilized them by releasing sperm directly into the water. On average, each female produced some 12,000 eggs in long strips surrounded by a protective, jellylike covering. The individual strings stretched for more than twenty feet and often became intertwined with the shallow vegetation and with eggs deposited by other females. Three days after the toads began to converge on the lake, egg laying was complete and they disappeared into the forests to feed and fatten up before hibernating again in the early fall.

During peak years at Lost Lake, we have seen more than 500 pairs of toads, several hundred unpaired males, and several million eggs strewn about the lake, but this year we counted only 147 breeding pairs and about 100 unpaired males. Almost two million eggs were laid. As usual, the eggs, with their jet-black embryos, began to develop normally, but two days after they were laid, we began to see the same ominous pattern we had observed for the past several years at lakes throughout the Cascades. Many embryos began to turn white as they started to die in wavelike fashion from one end of the enormous egg mass to the other. Soon they became a putrid, decaying mess, attracting flies and other insects—a potential feast that lured Pacific tree frogs (*Hyla regilla*) to the site. Opportunistic garter snakes arrived next to dine on the frogs. A week after the toad eggs had been laid, only half of them were viable. After the normal developmental period of about two weeks, even fewer hatched as tiny tadpoles. And this was only a small part of a much bigger problem.

By the mid-1980s, we began to notice that the frogs and toads we had been studying were becoming more difficult to find. Some populations of the most common species, such as the Cascades (*Rana cascadae*) and red-legged (*R. aurora*) frogs and the western toad, were nowhere to be found. Through the grapevine, I learned that amphibians were disappearing in many parts of the world, from North and South America, Asia, Africa, and Australia. Some species were even reported to have become extinct. To add to the mystery, in some areas populations of certain species were doing fine, while others were disappearing. In Oregon, for example, Pacific tree frog populations were thriving in lakes and ponds where western toad and Cascades frog populations were dwindling.

Biologists proposed many possible causes for the declines, including habitat destruction, pollution, and natural population fluctuations, but no single reason was apparent. In many areas, including our study sites, declines were occurring in relatively undisturbed habitat with no apparent pollution. Yet by the late 1980s, a pattern began to emerge that gave us some clues to the puzzling egg deaths—and perhaps to the shrinking populations—in the Cascades.

A significant number of the troubled species were mountain-dwelling amphibians that laid their eggs in the open, in shallow water—the same way in which the declining Oregon species did. Throughout development, these relatively unprotected eggs would be exposed to sunlight and potentially harmful ultraviolet (UV) radiation. The middle portion of the UV spectrum, known as UV-B, is especially dangerous. In humans it can cause sunburn and skin cancer and weaken the immune system. UV-B can also damage amphibians. In the mid-1970s, a labora-



tory study conducted by Robert Worrest, at Oregon State University, showed that western toad embryos developed abnormally when subjected to UV-B radiation. Since then, several reports have documented a gradual increase in UV-B radiation hitting the earth's surface as the protective ozone layer thins. Were increasing levels of UV-B radiation responsible for the high rate of egg mortality in the Cascades? And if other declining amphibians had a similar vulnerability at the egg stage, could increasing exposure to UV-B radiation each spring be responsible for shrinking their populations over time?

In the late 1980s, when I first suspected UV-B radiation caused the toad eggs to die, I began a series of simple experiments to see if this was possible. I brought some newly laid western toad eggs to my laboratory and reared them in the absence of sunlight in aquariums filled with lake water. I followed the eggs' development until they hatched. Several months later the tadpoles metamorphosed into young toads. I was excited to see that almost all of the laboratory eggs survived, while eggs from the same clutches left to develop in the lake had died at unprecedented rates. Whatever the problem was, it did not seem to be in the eggs themselves.

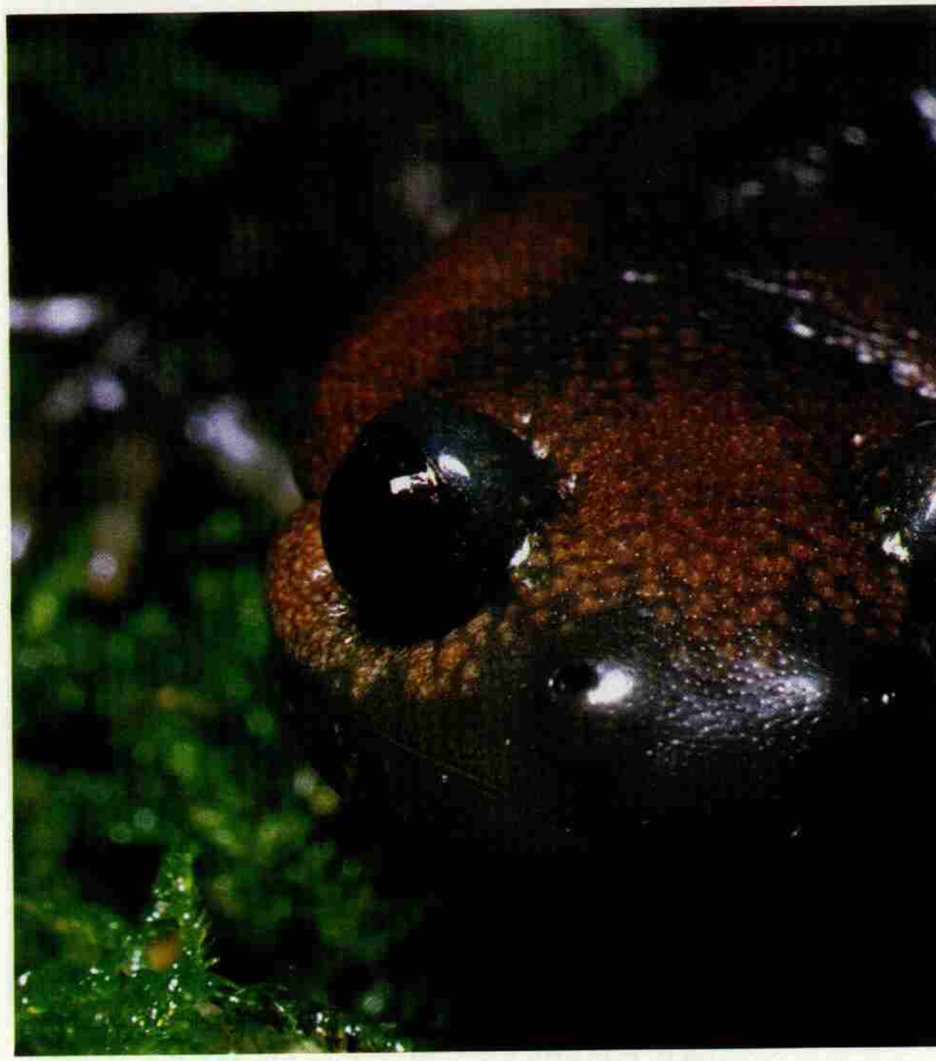
After hearing about my ideas on UV-B radiation and amphibian declines, John Hays, a molecular geneticist at Oregon State University, called me to discuss how he might get involved. To learn how plants and animals repair UV-B damage to DNA, Hays had been studying the process in the eggs of African clawed frogs (*Xenopus*)—the amphibian equivalent of the well-studied laboratory rat.

When a cell is exposed to UV-B radiation, the energy can be absorbed by a number of biologically important molecules, including proteins and DNA. When this happens, the bonds between atoms and molecules can be altered, causing the cell's chemical machinery to malfunction. Such changes are particularly disruptive when they occur in DNA; if the genetic code, which carries the instructions for life, is misread, mutations and cell death can occur. Many plants and animals, however, are able to repair a certain amount of DNA damage. Photolyase, an enzyme found in the cells of many organisms, can remove the harmful defects. Hays reasoned that the eggs of different amphibian species may contain different amounts of photolyase. Therefore, we predicted that species with the greatest quantities of the enzyme would be more resistant to damage by UV-B than species with less photolyase. This could explain why the eggs of some amphibians were dying while those of others were unaffected.

The first step in testing our hypothesis was to collect eggs from a number of amphibian species with different egg-laying behaviors. We also made sure to include eggs from species that were in decline and from species that were doing well. Once eggs were collected, Hays and his chief technician, Peter Hoffman, measured photolyase levels, using the same techniques they had perfected while studying African clawed frogs.

The results of the molecular tests were compelling. The eggs of the nine species we examined showed enormous differences in the amount of photolyase they contained. Eggs of the Pacific tree frog (which are laid in open, shallow water) had the most photolyase: three times as much as the Cascades frog and six times as much as the western toad. The eggs of the six salamanders we tested had less than any of the frogs, with the least amount being found in Dunn's salamander, *Plethodon dunni* (Pacific tree frog eggs had eighty times more photolyase than these amphibians).

The correlation between levels of photolyase and egg-laying behavior was striking. The salamanders, whose eggs have little photolyase, generally lay their eggs under logs, in crevices, or in deep water—all places where little UV-B radiation will penetrate—while species that lay their eggs in the open, exposed to sunlight, had the highest levels of the enzyme. The egg-laying behavior of salamanders may not have evolved specifically to afford them protection from UV-B radiation; other selective pressures, such as predation and temperature requirements for development may have been more important. Nevertheless, the protection from UV-B



may be a secondary benefit. Those species that laid their eggs in the open, however, needed high levels of photolyase to minimize the damage to their DNA caused by exposure to direct sunlight.

Although the results of the enzyme studies were suggestive, we still needed to know if UV-B radiation was damaging eggs in nature. We began tackling this question even before we had the results of the DNA repair study. With field experiments in lakes and ponds where amphibians naturally lay eggs, we could compare the hatching success of eggs exposed to UV-B with that of shielded eggs. We gathered freshly laid eggs from four species that deposited them in the open: Cascades frogs, Pacific tree frogs, western toads, and northwestern salamanders (*Ambystoma gracile*). We placed the eggs in the bottom of screened, boxlike enclosures that allowed water to flow freely through them. Over some of the enclosures, we placed plastic filters that blocked UV-B. We left a second set of enclosures uncovered, exposing the animals in them to the rays. A third set, which had a plastic filter that allowed transmission of UV-B, provided a control to insure that any variation we found under the UV-B-blocking model was not due to the presence of a plastic cover.

We placed the enclosures randomly in the shallow water of lakes or ponds where natural breeding sites were located. By using four enclosures of each type, we insured that our results were not caused by some bias in our procedure or by a small sample size. Setting up the experiments at several different sites helped assure that any results we obtained were not unique to a particular area.

Although we only had to follow the development of the eggs until they either hatched or died, the experiments took two years to complete. Like most fieldwork, the project ran into some unexpected trouble. During the first year, we could not get enough viable eggs to set up our experiments because the animals did not breed at all sites. Spring storms with high winds destroyed some of our enclosures. Under the same harsh spring weather encountered by the toads, we had to count and measure each egg, in every enclosure, every day. And then there was vandalism, both by humans and smaller mammals. So someone had to guard each site, twenty-four hours a day, until the experiments were done, which often took two weeks. By the end of the second year, however, we had results that were both dramatic and foreboding.

More than 40 percent of the western toad and Cascades frog eggs exposed to UV-B radiation died, compared with 10 to 20 percent of those that were shielded. Northwestern salamanders did not fare better; more than 90 percent of their exposed eggs died. The Pacific tree frog, however, was unscathed, with almost all of its eggs surviving under all lighting conditions.

These results, together with those from the DNA repair study, convinced us that the link between UV-B radiation and the egg deaths was real. Natural levels of UV-B were killing amphibian eggs in the field. Pacific tree frogs—which had the highest levels of photolyase and whose populations were doing fine—seemed more resistant to UV-B than did western toads and Cascades frogs, two species with less photolyase that are in decline throughout their ranges. We do not know the status of northwestern salamander populations, but given our results, they too could be in jeopardy.

We had found one small piece of the amphibian decline puzzle. But many questions remain. Is the egg mortality in the Cascades caused solely by UV-B exposure or are other factors involved? We had observed the growing presence of a pathogenic fungus in the lakes. Is the UV-B radiation compromising the defense systems of embryos, making them more susceptible to disease? Another question is, how much mortality during the egg stage can a population endure before it crashes? Western toads, for example, live for twenty years or more, so the results of their reproductive troubles in recent years may not become apparent for many years.

Finally, could we settle on a universal explanation for amphibian declines? In-

*Unlike most salamanders, the northwestern salamander often deposits its eggs in shallow water where ultraviolet rays penetrate.*
D. Grant Hokit

creasing levels of UV-B radiation are obviously not the only reason these animals were disappearing. It cannot explain, for example, why species that live under dense forest canopies, protected from UV-B, are also in trouble. Perhaps other organisms, such as plants, fish, insects, and even humans will provide us with more information on the damaging effects of increasing levels of UV-B. We know, for example, that certain crop plants have reduced growth, photosynthetic activity, and flowering when exposed to UV-B radiation. In the Antarctic, severe ozone depletion and increased UV-B have been associated with reduced growth in phytoplankton.

If projected increases in UV-B occur, over evolutionary time there may be increased selection pressure on amphibians and other organisms to evolve efficient repair mechanisms or to alter their behaviors and thereby minimize their exposure to UV-B. Unfortunately, changes wrought by human disturbance occur at such rapid rates that many organisms may not have time to adapt. □

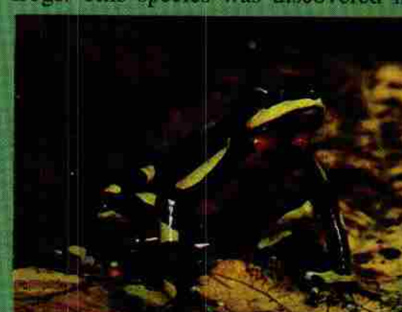
## Here Today, Gone Tomorrow?

The impact of human activities on amphibians is difficult to gauge. Natural fluctuations in their populations and the lack of long-term data on their numbers have led to some uncertainty about their status. Nevertheless, an alarming number of amphibians are on the endangered list. Their semiaquatic life styles make amphibians particularly vulnerable to change; habitat destruction, which is probably the single most important cause for the decline of most species, affects amphibians both on land and in the water where they breed. Their permeable skin readily absorbs waterborne pollutants, they have no hair or feathers for protection, and their eggs are not encased in hard shells. In addition to habitat loss, a host of other factors—disease, water acidification, increased UV-B radiation, and introduced species in ponds and lakes—may be contributing to dwindling amphibian populations.

Golden toad (*Bufo periglenes*)
Gregory G. Dimijian; Photo Researchers, Inc.

Red-legged frog (*Rana aurora*)
Frank Schneidermeyer; Oxford Scientific Films

Some of the more attractive and interesting toads and frogs are among the amphibians that are disappearing. Populations of the red-legged frog (*Rana aurora*), found from British Columbia to northern Baja California, have dwindled drastically in California and Oregon, but the cause of the decline is unknown. Australia's gastric brooding frog (*Rheobatrachus silus*) is one of the most fascinating amphibians because of its habit of brooding its offspring in its stomach and then expelling them as tadpoles or young frogs. This species was discovered in 1973 and has not been seen in nature since 1979. The golden toad (*Bufo periglenes*), seen here mating, is found only in Costa Rica's Monte Verde Cloud Forest Preserve; its range is a few miles along the crest of the continental divide. This diminutive toad was abundant in 1987, but is now extremely rare. The harlequin frog (*Atelopus varius*) has vanished from the same area.—*R. A.*



Harlequin frog (*Atelopus varius*)
Michael Fogden; Oxford Scientific Films

Australian gastric brooding frog (*Rheobatrachus silus*)
R. W. G. Jenkins; NHPA