Explaining Frog Deformities

An eight-year investigation into the cause of a shocking increase in deformed amphibians has sorted out the roles of three prime suspects

ONE HOT SUMMER DAY in 1995 eight middle school children planning a simple study of wetland ecology began collecting leopard frogs from a small pond near Henderson, Minn. To their astonishment, one captured frog after another had five or more hind legs, some twisted in macabre contortions. Of the 22 animals they caught that day, half were severely deformed. A follow-up search by pollution-control officials added to the gruesome inventory. Occasional frogs in the pond had no hind limbs at all or had mere nubbins where legs should be; others had one or two legs sprouting from the stomach. A few lacked an eye.

The story seized national media attention and raised many questions—among them, was this an isolated occurrence or one facet of a widespread trend? And what caused the deformities? As researchers elsewhere in the country began investigating their local amphibian populations, it became clear that this bizarre collection of ailments was not confined to Minnesota. Since 1995, malformations have been reported in more than 60 species, including salamanders and toads, in 46 states. In some local populations 80 percent of the animals are afflicted. International reports show that this phenomenon extends beyond the U.S. Surprising numbers of deformed amphibians have been found in Asia, Europe and Australia as well. Worldwide, extra legs and missing legs are most common.

The aberrations cannot be discounted as being a normal part of amphibian life. Research dating back to the early 1900s indicates that a few individuals in every population have defects resulting naturally from genetic mutation, injury or developmental problems. In healthy populations, however, usually no more than 5 percent of animals have missing limbs or digits; extreme deformities, such as extra hind legs, are even less common. Moreover, fresh reviews of historical records by one of us (Johnson) and new field studies indicate that deformities have become more prevalent in recent times.

Over the past eight years, dozens of investigators have blamed the increase on the amphibians' greater exposure to ultraviolet radiation, on chemical contamination of water or on a parasite epidemic. Not surprisingly, every time another report appeared, media outlets touted the new view, thus providing a misleading picture of the situation. It turns out that all these factors probably operate to varying extents, each causing particular disfigurements, and that all three may at times act in concert. Moreover, all stem in part from human activities such as habitat alteration.

Deformities undoubtedly impair amphibian survival and most likely contribute to the dramatic declines in populations that have been recognized as a global concern since 1989 [see "The Puzzle of Declining Amphibian Populations," by Andrew R. Blaustein and David B. Wake; SCIENTIFIC AMERICAN, April 1995]. Both trends are disturbing in their own right and are also a warning for the planet [*see box on page 63*]. Amphibians have long been regarded as important indicators of the earth's health because their unshelled eggs and permeable skin make them extremely sensitive to perturbations in the environment. Chances are good that factors affecting these animals harshly today are also beginning to take a toll on other species.

An Early Suspect

ONE PUTATIVE CAUSE of the deformities, excess exposure to ultraviolet radiation, came under suspicion almost as soon as the malformations were discovered, because it had already been implicated in declines of amphibian populations and because laboratory work had shown it to be capable of disrupting amphibian development. This form of radiation—which can damage immune systems and cause genetic mutations, among other effects—has been reaching the earth in record doses since chlorofluorocarbons and other human-made

ALARMING LEGS: Ecologist Andrew R. Blaustein eyes malformed Pacific tree frogs collected from farm ponds in northwestern Oregon.

FRANS LANTING *Minden Pictures*

BY ANDREW R. BLAUSTEIN AND PIETER T. J. JOHNSON

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chemicals began thinning the protective layer of ozone in the stratosphere, a problem first measured in the 1970s. Between 1994 and 1998 one of us (Blaustein) and his colleagues demonstrated that exposure to ultraviolet rays can kill amphibian embryos and larvae, cause serious eye damage in adult frogs, and induce various types of bodily deformities in frogs and salamanders.

Whether exposure to ultraviolet radiation could disrupt leg development remained uncertain until the late 1990s, when Gary Ankley and his co-workers at the Environmental Protection Agency in Minnesota carried out the most focused experimental research on this question to date. When the investigators shielded developing frogs from ultraviolet rays, the animals grew normal limbs, whereas tadpoles exposed to full doses of natural levels of ultraviolet radiation developed with parts of their legs missing or without digits. These deformities resembled some of those found in wild frogs from several sites around the country.

The EPA team was quick to point out, however, that ultraviolet radiation does not explain all types of leg deformities seen in nature. Most notably, it does not lead to the growth of *extra* legs, one of the deformities reported most frequently since 1995. Many laboratory and field experiments, several performed by Blaustein and his colleagues, have come to the same conclusion. Other biologists have also pointed out that many wild amphibians can avoid the continuous exposure to radiation studied in the EPA experiments. Juvenile and adult amphibians alike can move in and out of sunlight, often live in muddy water, or may be nocturnal.

Pollution's Part

AS SOME RESEARCHERS were examining the link between ultraviolet radiation and deformities, others were pursuing the influence of water pollution, such as pesticide runoff. They focused on pollution because so many of the early reports of amphibian ailments came from areas where large amounts of insecticides and fertilizers are applied every year. By the mid-1990s numerous laboratory studies had shown that myriad contaminants can kill amphibians, but it was unclear whether they could induce extra or incomplete limb formation.

A major challenge for toxicologists is isolating a single chemical or even a group of chemicals as a likely candidate. Millions of tons of hundreds of different pollutants are applied annually in regions where deformed amphibians have been found. Yet one chemical rose immediately to the top of the list: methoprene. First approved for commercial use in 1975, methoprene was promoted as a safer replacement for the banned pesticide DDT.

Initial concern over methoprene came from its chemical similarity to compounds called retinoids. These substances, especially retinoic acid, play an integral role in vertebrate development; too little or too much can lead to deformities in embryos. Indeed, numerous miscarriages and birth defects in humans have resulted from pregnant women's use of acne medicines that contain a retinoic acid derivative.

Some biologists suspected methoprene might have a similar effect on frogs. In a series of experiments in the late 1990s, the EPA in Minnesota did show that high amounts of retinoic acid could trigger poor formation of hind limbs in frogs, but comparable tests with methoprene caused no malformations at all. Separate field measurements also indicated that the pesticide could not be the sole cause. Methoprene breaks down quickly in the environment, and investigators found little evidence that it persists where deformities are abundant. The same is true for 61 other agricultural chemicals and their breakdown products that have been measured in locations that harbor malformed animals throughout the western U.S. Pesticides are not off the hook, however. Hundreds remain untested, and some evidence implies that certain pesticides can cause bodily damage (albeit not the formation of extra limbs).

At the moment, then, laboratory research tentatively suggests that water pollutants and ultraviolet radiation are capable of causing disfigurement. But a more potent threat appears to have a much broader impact in nature.

Prolific Parasites

THE EARLIEST HINTS of this threat—apparently the cause of the widespread hind-leg anomalies—turned up long before the disturbing findings in Minnesota garnered nationwide attention. In the mid-1980s, Stephen B. Ruth, then at Monterey Peninsula College, was exploring ponds in northern California when he found hundreds of Pacific tree frogs and long-toed salamanders with missing legs, extra legs and other deformities. He sounded no alarm, however, because he assumed he was seeing an isolated oddity.

In 1986 Ruth asked Stanley K. Ses-

Overview/*Amphibian Ailments*

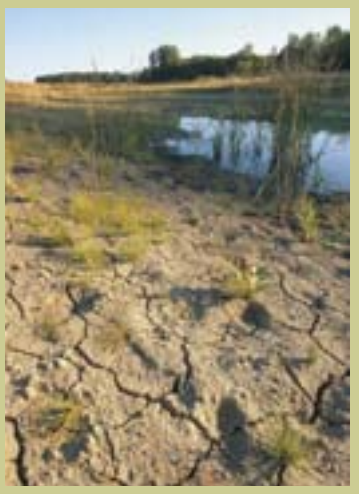
- Since the mid-1990s striking deformities have turned up in more than 60 species of frogs, toads and salamanders in 46 states and on four continents. The number of disfigured animals in some populations averages around 25 percent—significantly higher than in previous decades.
- Contradictory reports have blamed the deformities on increasing exposure to ultraviolet radiation, contaminated water or a parasite epidemic.
- New evidence indicates that the parasite epidemic accounts for one of the most prevalent deformities—extra hind legs—and strongly suggests that human activities such as habitat alteration are exacerbating the problem.

Disfigured and Dwindling

Do the deformities explain recent declines in amphibian populations?

FROGS, TOADS AND SALAMANDERS have been climbing up the long list of creatures in danger of disappearing from the earth entirely ever since the first reports of dwindling populations were made 20 years ago. An obvious question for biologists is to what degree physical deformities are contributing to overall population declines.

Most malformed amphibians eventually vanish from a population because they can neither escape their predators nor hunt for food efficiently. Events known to increase the number of animals that mature into disabled adults—such as the parasite epidemic that is currently afflicting dozens of sites across North America—could cause a whole population to crash, particularly if the incidence of deformities continues to increase. Although such crashes may be occurring at some sites, numerous amphibian populations have declined severely in the absence of any deformities, leaving researchers to conclude that deformities are far from the sole basis for the declines. Environmental hazards seem to be a more significant cause.



CRACKED EARTH signals the temporary demise of a frog habitat in the U.S. West.

Amphibian species inhabit a wide variety of ecosystems, including deserts, forests and grasslands, from sea level to high mountains. But as diverse as their niches are, few are shielded completely from a nearly equal variety of insults that humans inflict on them. Some important amphibian habitats have been totally destroyed or are polluted to an intolerable degree. In other cases, people have introduced foreign animals that either devoured or pushed out the native amphibians.

Some of the most widespread alterations may lead to both population declines and deformities. Many studies have shown, for instance, that excess ultraviolet radiation—resulting from human-induced ozone loss in the upper atmosphere—can inhibit limb formation in amphibian juveniles or even kill embryos inside their vulnerable, unshelled eggs. In the future, global warming is expected to dry out certain suitable aquatic habitats while elsewhere encouraging the emergence of infections that produce abnormal development. When it comes to problems as pressing as these, tackling declines will most likely help alleviate the deformities as well. *—A.R.B. and P.T.J.J.*

sions, now at Hartwick College in New York State, to inspect his bizarre amphibians. Sessions agreed and quickly realized that they were all infected with a parasitic trematode, known commonly as a flatworm or a fluke. The California trematodes—whose specific identity was unknown at the time—did not appear to kill their hosts outright, but Sessions suspected that their presence in a tadpole mechanically disturbed natural development wherever the parasites formed cysts in the body, most frequently near the hind legs. To test this hypothesis, he simulated trematode cysts by implanting small glass beads in developing limb buds of African clawed frogs and a salamander known as an axolotl. These two species, which serve as the "white rats" of amphibian biology because they are easy to breed in captivity, developed extra legs and other abnormalities—much as if they were parasitized.

As intriguing as those experimental results were, though, they could not prove that trematodes were responsible for the deformities in Ruth's specimens: African clawed frogs and axolotls are not known to have limb deformities in nature. The research that would eventually connect the dots between trematodes and extra or missing legs in frogs was conducted after the stir of 1995. At that time, Johnson pored over the scientific literature for clues to the cause and came across the discoveries made by Ruth and Sessions. Johnson and his colleagues then conducted broader surveys of California wetlands between 1996 and 1998 and discovered that ponds where tree frogs had abnormal limbs also had an abundance of the aquatic snail *Planorbella tenuis*, one in a series of hosts colonized by Sessions's trematode, now known to be *Ribeiroia ondatrae*.

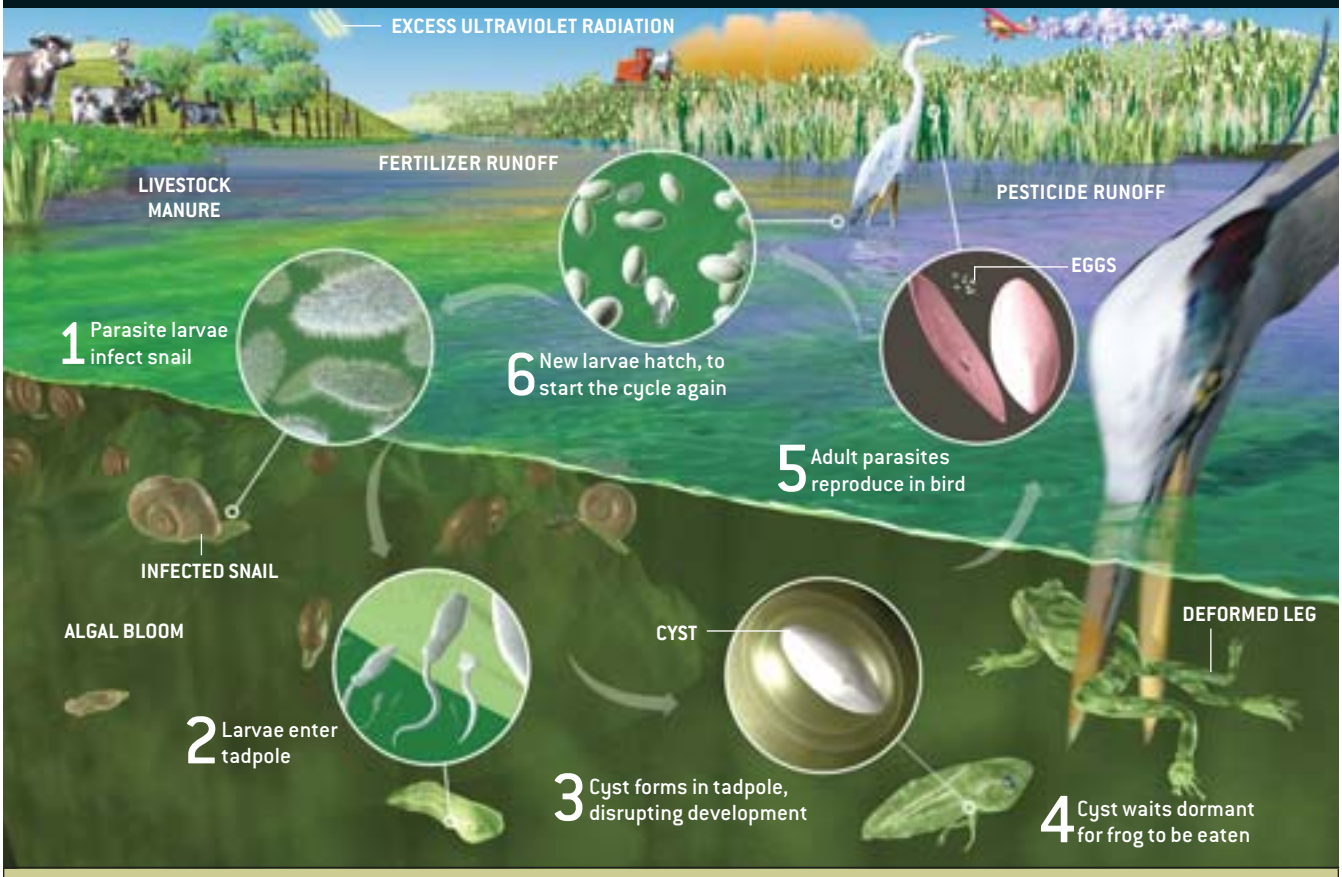
Thinking that they may well have uncovered a direct correlation between a parasite epidemic and amphibian deformities in the wild, Johnson and his team immediately collected deformed frogs from the same ponds and dissected them. In every case, they found cysts of the parasite densely clustered just below the skin around the base of the hind legs. To test the idea that the trematodes were triggering the growth of the extra limbs, the researchers then exposed Pacific tree frog tadpoles to *R. ondatrae* parasites in the lab. As expected, the infected tree frogs developed deformities identical to those

THE AUTHORS

ANDREW R. BLAUSTEIN and *PIETER T. J. JOHNSON* began exploring the potential causes of amphibian deformities as a team in 1998. Blaustein, who earned a Ph.D. in 1978 from the University of California, Santa Barbara, is professor in the zoology department at Oregon State University. A behavioral and population ecologist by training, he has spent the past several years investigating the dynamics of worldwide declines in amphibian populations, specifically addressing the effects of ultraviolet radiation, pollutants, pathogens and nonnative species. Johnson, a doctoral candidate at the Center for Limnology at the University of Wisconsin–Madison, studies human influences on emerging diseases in aquatic environments.

FRANS LANTING *Minden Pictures*

HOW PARASITES CAN CRIPPLE FROGS



LIFE CYCLE of the trematode *Ribeiroia ondatrae* enables the parasite to induce deformities—including extra hind legs—in generation after generation of frogs. In its first larval form the trematode infects snails (*1*). After transforming into a second free-swimming form inside a snail, the parasite embeds itself near a tadpole's future hind leg (*2*). There it forms a cyst that disrupts normal limb development and can cause the tadpole to sprout extra legs as it grows into a frog (*3*). The disabled frog then becomes easy prey for the parasite's final host, often a heron or egret (*4*). The parasite matures and reproduces inside the bird, which releases trematode eggs into the water with its feces (*5*). When larvae hatch (*6*), they begin the cycle again. Human activities can exacerbate this process, especially where livestock manure or fertilizers enter a pond and trigger algal blooms that nourish, and thus increase, snail populations. Excess ultraviolet radiation and pesticide runoff—which might cause other types of deformities when acting alone—may facilitate the cycle by weakening a tadpole's immune system and making the animal more vulnerable to parasitic infection.

—A.R.B. and P.T.J.J.

found in nature, including extra limbs and missing limbs. Higher levels of infection led directly to more malformations, whereas uninfected frogs developed normally.

This study turned out to be a key breakthrough in solving the mystery of deformed amphibians. Subsequent experiments, including one we conducted in 2001 on western toads, provided evidence of *Ribeiroia*'s major role in disfiguring amphibians other than Pacific tree frogs. Two studies reported last summer by Joseph M. Kiesecker of Pennsylvania State University and by a team made up of Sessions, Geffrey Stopper of Yale University and their colleagues showed that *Ribeiroia* can cause limb deformities in wood frogs and leopard frogs as well.

Other evidence indicates that *Ribeiroia* is almost always found where deformed amphibians are present, whereas chemical pollutants are found much less frequently. What is more, the parasitic infection seems to have skyrocketed in recent years, possibly reaching epidemic levels. An exhaustive literature search we conducted early in 2001 identified only seven records prior to 1990 of amphibian populations that exhibited both significant malformations and *Ribeiroia* infection.

In contrast, a field study that we published last year turned up 25 such habitats in the western U.S. alone. Among those sites, six species displayed deformities, and the proportion of affected individuals in each population ranged from 5 to 90 percent. Over the past two years, other investigators have identified *Ribeiroia*-triggered deformities in Wisconsin, Illinois, Pennsylvania, New York and Minnesota, including the pond where the eight schoolchildren made headlines. Although heightened surveillance could account for some of this increase in report-

DAVID FIERSTEIN

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ing, the vast majority of deformed frogs have been found by people, often children, who were looking for frogs for reasons unrelated to monitoring abnormalities.

Not Working Alone

SCIENTISTS NOW UNDERSTAND how the life cycle of *Ribeiroia* helps to perpetuate the development of deformities in generation after generation of amphibians that are unlucky enough to share a habitat with infected snails [*see illustration on opposite page*]. After the parasite leaves its snail host and enters a tadpole, it embeds itself near the tadpole's hind leg. Infected tadpoles then sprout extra legs or fail to develop both limbs. In either case, the young amphibian becomes unable to move properly and thus becomes easy prey for the parasite's final host, often a heron or egret. The parasite matures inside the bird and becomes reproductively active. Through the bird's feces, trematode eggs enter the water. When the larvae hatch, they find a snail and begin the cycle again.

If a spreading epidemic of *Ribeiroia* accounts for much, or even most, of the increase in frog deformities seen in recent years, what accounts for the epidemic? Current environmental trends suggest that human alteration of habitats is at fault. In human as well as wildlife populations, infectious diseases emerge or become more prevalent as features of the landscape change in ways that favor the proliferation of disease-causing organisms. Reforestation of the northeastern U.S., for example, has led to the emergence of Lyme disease by encouraging the proliferation of white-tailed deer, which transport ticks that harbor the Lyme bacterium. On the other side of the Atlantic, the damming of African rivers has led to the spread of human blood flukes that depend on snails as a host and cause human schistosomiasis. During the past several decades, alteration of habitats has also encouraged the expansion of such diseases as hantavirus, Ebola, West Nile virus, dengue fever and AIDS.

DEFORMITIES DIFFER in their most likely causes. Trematode parasites can trigger the growth of extra hind legs (*left*). They can also lead to poorly formed or missing hind legs, although excess ultraviolet radiation, chemical pollutants or injury from predators may also be to blame. Ultraviolet radiation probably accounts more often for abnormalities of the eyes (*right*) and skin.

We recently showed a direct relation between human habitat alteration and sites where *Ribeiroia* parasites are especially abundant. Indeed, our survey of the western U.S., reported in 2002, revealed that 44 of the 59 wetlands in which amphibians were infected by *Ribeiroia* were reservoirs, farm ponds or other artificial bodies of water. Fertilizer runoff and cattle manure near these habitats often encourage overwhelming blooms of algae, which means more food for the snails that host *Ribeiroia* parasites. Larger populations of snails infected with *Ribeiroia* lead directly to more deformed frogs. Wading birds, the other necessary parasite hosts, are usually found in abundance at such human-made locales.

Although parasitism by trematodes is the likeliest explanation for most outbreaks of amphibian deformities, it is certainly not the only cause and may often be abetted by additional factors. At times, water pollutants or excess ultraviolet radiation may act alone to cause specific problems, such as disfigured bodies and eye or skin abnormalities. At other times, pollutants or radiation may set the stage for infection by weakening an amphibian's immune system and thus leaving the animal more vulnerable to a parasitic invasion. In yet another scenario, an increase in amphibian predators, such as fish, leeches or turtles, may create more deformities by biting off tadpole limbs.

Clearly, amphibians are subjected to a cocktail of agents that stress individual animals and then, perhaps, entire populations. The challenge to scientists becomes teasing apart these agents to understand their interactions. Humans and other animals may be affected by the same environmental insults harming amphibians. We should heed their warning. SA

MORE TO EXPLORE

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Visit the North American Reporting Center for Amphibian Deformities at **www.npwrc.usgs.gov/narcam/**

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