Massive frog die-offs have for years been linked to environmental conditions, but new data from Australia suggest that the real killer may be a deadly fungus

Are Pathogens Felling Frogs?

TOWNSVILLE, QUEENSLAND, AUSTRALIA—On a Tuesday morning last August, Ken Aplin, curator of reptiles and amphibians at the Western Australia Museum in Perth, got a chilling phone call. The owner of a nearby organic nursery wanted to know why hundreds of frogs—common motorbike frogs, whose call sounds like a motorcycle changing gears—had suddenly died on his chemical and pesticide-free grounds. Aplin didn't have a ready answer, but he feared the worst. A few weeks later, his suspicions were confirmed: A colleague tested a freshly dead motorbike frog from another nearby source and found that the animal was lethally infected with a parasitic chytrid fungus—a virulent amphibian pathogen that had caused sudden, massive die-offs of more than a dozen frog species here in Queensland; four species have apparently gone extinct. Now, it seemed, the fungus had leaped 6000 kilometers and across the dry Nullarbor Desert, spreading to western Australia, a region with a rich endemic frog fauna that had never seen massive die-offs before.

FROG DECLINES

New data suggest that pathogens are causing die-offs and deformities in wild frogs. One News story highlights a fungus thought to kill frogs, while another one (p. 731) and two Reports (pp. 800 and 802) describe how a different parasite may cause some frog deformities.

▶ DIE-OFFS
DEFORMITIES

"It was like hearing about a first case of cholera," says Aplin. "I feared it would spread." In the next few months, the disease was found on other dead frogs in Perth and nearby towns to the south, often killing every frog in backyard ponds. "I suspect that we're on the edge of a major outbreak that will cause mass mortalities in the next few months, when frogs gather to breed," he adds.

Australia considers itself the front line in dealing with this frog pathogen, *Batrachochytrium dendrobatidis*, a new genus and species described as a lethal disease 9 months ago (*Science*, 3 July 1998, p. 23) and named only last month. But the chytrid is not just an Australian problem. It is suspected in the catastrophic disappearance of frogs in Panama and Costa Rica; and it is implicated in mass die-offs in the United States as well. Indeed, some researchers—mostly epidemiologists—say that this virulent new fungus may be the key factor in the sudden, mysterious decline of frogs around the globe, particularly those from wilderness areas of the Americas and Australia.

Since the 1970s, populations and species of frogs have been vanishing worldwide, and deformities such as missing legs have been turning up with alarming frequency, sparking massive research and monitoring programs. In many cases, frog populations or even entire species in pristine, remote mountain areas suddenly vanished in a few months. Baffled about why frogs in protected areas would be so vulnerable, many researchers have looked to the global environment, arguing that frogs are like canaries in a coal mine, serving as indicators of global ecological health (see sidebar). They have studied a plethora of environmental suspects, ranging from increased ultraviolet (UV) light to global warming and wind-borne pollutants. Yet despite almost a decade of intense research and some loose correlations between die-offs and environmental factors, no one so far has been able to show that these factors are actually killing frogs.

Motorbike frog run over? A fungus killed western Australia's motorbike frog by the hundreds.

Thus, when the chytrid was first fingered, many scientists regarded it as just one of many "smoking guns" that would prove less convincing as time went by. But this time, researchers have bodies to prove the case. The Australian experience has galvanized researchers there, and now scientists elsewhere are taking seriously the idea that the chytrid plays a central role in the declines. A team of U.S. researchers has just proven in the lab that the chytrid alone can kill healthy frogs. And by studying preserved specimens, other researchers have now implicated the fungus in some of the very die-offs that first raised the amphibian alarm in the United States, including mass deaths of leopard frogs, *Rana pipiens*, in the Colorado Rockies back in 1974, and in more recent disappearances of Arizona's lowland leopard frogs. "It's increasingly clear that we need to treat the chytrid—and amphibian diseases in general—as a serious threat," says Cynthia Carey, a physiological ecologist at the University of Colorado, Boulder. "Diseases are killing frogs and we need to know why."

Still, when it comes to worldwide frog declines, several leading U.S. herpetologists, such as David Wake of the University of California, Berkeley, resist the notion of a single cause; others note that some chytrid-infected frogs survive. So researchers are working to test two competing ideas: that the chytrid is an emerging pathogen sweeping through previously unexposed populations, or that an environmental cofactor such as increased UV light or climate change is magnifying the chytrid's effects. "I don't think you can rule out" such cofactors, says Donald Nichols, a pathologist at the National Zoo in Washington, D.C., who first identified the disease. He and others hope that a flurry of chytrid research will help them find out.

Death down under

The story down under starts here in northeastern Australia, where herpetologists began monitoring frog populations in 1989 after several species dwindled or disappeared from some of the least touched places on Earth, including World Heritage rainforest parks in the Atherton Tablelands. At first, environmental pollutants were thought to be

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Frogs: Canaries in The Hot Zone?

Back in 1990, when 40-odd herpetologists and ecologists convened in Irvine, California, to discuss the mysterious problems afflicting frogs, they concluded that frog declines were symptomatic of larger environmental problems on the planet. Like the canary that miners carried to warn them of bad air, frogs could also warn us that "something is desperately wrong with our environment," says ecologist Andrew Blaustein of Oregon State University in Corvallis.

But now evidence is mounting that the frog die-offs—and the disturbing limb deformities seen in some populations—may be due in part to a different class of problem: pathogens. A lethal chytrid fungus is the primary suspect in many massive frog die-offs (see main text), and in this issue, researchers report that a snail-borne parasite can cause some of the extra and missing limbs seen in wild frogs (pp. 731, 800, and 802). If so, frogs' plight may not be indicative of the ills afflicting Earth as a whole, at least not in the way researchers first thought.

The original idea was that frogs, because of their diverse biology—they live both in water and on land, are vegetarians as tadpoles and carnivores as adults, and have permeable, unprotected skin—are more sensitive to environmental changes than such species as birds, explains herpetologist George Rabb, director of Chicago's Brookfield Zoo. The "canary in a coal mine" idea took hold, and this status was a boon to frog research, as granting agencies funded studies on environmental ills such as wind- and waterborne pollutants, ultraviolet (UV) light, and global warming. Just 2 months ago, Interior Secretary Bruce Babbitt asked Congress for $8.1 million to set up a task force to look into frog declines and deformities.

After a decade of work, researchers have shown that environmental factors such as global warming are sometimes correlated with die-offs, and that some of these factors can stress frogs in the lab. But no one has been able to show conclusively that an environmental trigger is responsible for the major, sudden frog declines.

Still, some researchers argue that even if pathogens are to blame for some frog disorders, the amphibians can still be considered early warning signals for environmental problems. "Absolutely yes," says Blaustein. He and other herpetologists argue that factors such as UV light and global warming do stress frogs, and that the chytrid may simply pick off amphibians weakened by these factors. They insist that there will always be many reasons for the die-offs. "Maybe in some places, a disease is going to kill the frogs; elsewhere it's going to be habitat loss, or herbicides, or UV light. It's not going to be a simple, one-size-fits-all story," says Blaustein.

Other researchers admit that having an amphibian pathogen as the villain takes some of the shine off the frog as poster child for the entire environment. However, that doesn't mean that the environment can be ignored, cautions Richard Speare, a wildlife infectious disease specialist in Australia. "The chytrid's presence strengthens the need to have optimal environments to tip the balance in favor of the frog," he says.

Speare and others add that if the brisk intercontinental trade in frogs does help spread the chytrid fungus, as some researchers suspect, then frogs may be sending another message about humans' influence on the environment. "Maybe emerging wildlife diseases warn us of a different global environmental threat: the introduction of [wildlife] diseases on a global scale," says Speare's colleague, Peter Daszak, a parasitologist at the University of Georgia, Athens. "This could be just as significant a threat to the global environment as other forms of anthropogenic change—and one which is far more difficult to correct." **–V.M.**

the cause. But the streams where the frogs died aren't polluted, and the amount of UV light here hasn't risen during the past few decades. In this case, "you can rule out any of these environmental cofactors," says Richard Speare, an infectious disease specialist at James Cooke University here. And infected frogs don't show "multiple opportunistic infections, such as you'd expect if their immune systems were compromised," notes parasitologist Peter Daszak of the University of Georgia, Athens, who identified the chytrid in Australian frogs.

The pattern of death "has all the hallmarks of an emerging pathogen," says Speare, particularly its ability to infect a broad range of animals; thus it can continue to spread even after wiping out one species entirely. In Australia, researchers have found the chytrid on almost every suffering frog population they have checked, and they have been able to chart the fungus's spread through the continent, starting at the northern end of the epidemic in Queensland in 1993. So far they've traced it 300 kilometers south and 4 years back to 1989. The very first die-offs struck just north of the port of Brisbane in 1979, so Speare speculates that the fungus was introduced to Australia in the late 1970s, perhaps on an exotic frog.

Traveling at about 100 kilometers a year, the chytrid decimated many species, moving "like a wave" through frog populations in successive localities. Last year it apparently crossed the continent to Perth and western Australia, perhaps on the feet of an infected frog that stowed away in a box of fruit destined for Perth. "That happens all the time," says Aplin. "A store clerk opens a box of bananas from Queensland and out hops a frog"—and with it the chytrid. Such a scenario would explain why the first cases in southwestern Australia appeared in urban areas.

At risk. Australia's western spotted frog may be hit by the chytrid.

CREDIT: GREG HAROLD

Not all Aussie frogs have suffered equally from the chytrid: Queensland's green-eyed treefrog, which died from the disease in great numbers in the late 1980s and early '90s, is slowly coming back, suggesting that this species may have some resistance. But four stream-dwelling species, including two unique gastric-brooding frogs, are presumed to have been wiped out by the fungus. It has also infected another 24 species and drastically shrunk the population of 11 of these.

At the same time the Australians were identifying the pathogen, researchers in Central America were puzzling over their own frog deaths. After hearing about the Australian situation, researchers checked for the chytrid—and found it on 10 different dead frog species in western Panama. The chytrid is also linked to the disappearance of numerous species in the rainforests of Costa Rica, says herpetologist Karen Lipps of Southern Illinois University in Carbondale.

Preliminary genetic work suggests that the Central American frog fungus is the same one that plagued Aussie frogs. David Porter from the University of Georgia, Athens, chytrid specialist Joyce Longcore from the University of Maine, Orono, and graduate student Timothy James from Duke University in Durham, North Carolina, have found that the 18*S* ribosomal DNA genetic regions of chytrids are nearly identical, implying that a

single fungal species is sweeping into new realms worldwide. James also notes that it is "quite an odd fungus," different from the chytrids found commonly in soil and water. "There's no doubt we're seeing a new, emerging disease, one that is highly pathogenic and hits a wide range of amphibians," concludes Daszak. Further molecular data should reveal how recently the chytrid has spread through frogs on various continents, and perhaps whether it has newly evolved to attack amphibians or if it is an old frog nemesis now invading naïve populations.

Some researchers haven't been sure that the chytrid can kill healthy frogs. But a few months ago, in as-yet-unpublished work, a team led by the National Zoo's Nichols isolated the chytrid from poison dart frogs killed by the disease. They then inoculated healthy frogs with this chytrid. All infected frogs died, whereas frogs inoculated with a placebo did not. Next, they reisolated the chytrid from the second batch of dead frogs, a sequence of experiments that fulfills what are called Koch's postulates, the gold standard for proving that an organism causes a disease.

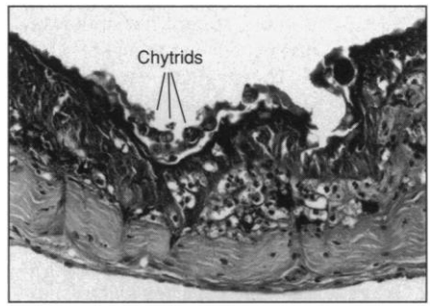
The chytrid apparently uses the keratin in the frog's skin as a nutrient. Its motile, water-borne spores invade surface skin cells and grow and divide there asexually. No one is quite sure just how it kills frogs; Speare suspects that the fungus secretes a toxin, as dying frogs are unable to keep their balance and seem to have seizures, whereas others think the fungus blocks water uptake. In Australia, species hit hardest tend to spend most of their lives in water and live at higher and cooler altitudes, says Speare. That fits with both Australian and American lab studies showing that the chytrid is hard to grow at above 30°C and dies without water.

Winter kill. Arizona's lowland leopard frogs vanished in winter.

Revisiting U.S. die-offs

The chytrid's cold, wet-loving habits may help explain dramatic frog deaths in the United States as well, some researchers say. Take the decline of the once-common lowland leopard frog in Arizona. Over the last decade, researchers have pointed their fingers at the usual list of suspects, including loss of wetlands, heavy metals, and bacterial infections. But no one had ever seen wild frogs dying en masse from these killers. Scientists only knew that when they returned to the field each season, fewer and fewer frog populations were left.

Then, in January of this year, Michael Sredl, a herpetologist at the Arizona Game and Fish Department in Phoenix, spotted a leopard frog population north of Phoenix in its death throes: The frogs were emaciated, trembling, and rigid. They had no obvious skin lesions, ulcers, or fungal growth, but histological sections of their skin revealed lethal numbers of the chytrid and its spores.



On the attack. Chytrids invade a toad's skin.

This sighting also taught Sredl something else: Frogs were dying in winter. "We'd missed it every other year, because herpetologists usually don't go looking for their animals in the winter," he says. The chytrid has now been "positively implicated" in die-offs of two leopard frog species in Arizona, as well as a species of treefrog, says Sredl. It's "under investigation in the declines of all Arizona ranid frogs." And it's been found in specimens collected last year of Pacific treefrogs and endangered mountain yellow-legged frogs from California's High Sierras. Other scientists believe the chytrid may be responsible for a slew of other die-offs as well, including the extinction of *Rana pipiens* and *Bufo boreas* from the Colorado Rocky Mountains in the 1970s, a '70s crash of the *Rana pipiens* population around the Great Lakes, and sudden die-offs of ranids and toads from Wyoming to New Mexico in the 1980s.

Pathogens have been suggested as culprits before. Back in 1993, Norman Scott, a zoologist at the U.S. Geological Survey in San Simeon, California, suggested that a novel pathogen was killing western frogs. He named the disease the "postmetamorphic death syndrome," because newly metamorphosed frogs seemed to die overnight. That's a characteristic of chytrid infection, because tadpoles carry the disease only in their mouths and survive; after metamorphosis, the fungus spreads throughout the frog's skin and kills it. But Scott's idea received little attention at the time, perhaps, he says, because researchers were so determined to find an environmental cause.

Now U.S. scientists are gearing up to have both historical and freshly collected specimens checked for the fungus, something the Australians began a year ago. "Only a week ago, I would never have thought to do this—collect frogs for testing," says Michael Lannoo, a herpetologist at the Indiana University School of Medicine in Muncie, who recently realized that the pattern of declines he's studying in the Midwest's northern cricket frog matches that of earlier chytrid die-offs.

However, even the most avid defenders of the chytrid hypothesis say it's not responsible for every decline. The fungus has not been found in Europe, for example. And herpetologists generally agree that the biggest problem for frogs worldwide is habitat loss; a species with only a few small, fragmented populations is likely to be much more vulnerable to the chytrid or another disease. "When you turn a diverse ecosystem into a hog lot," notes Val Beasely, a veterinary toxicologist at the University of Illinois, Urbana-Champaign, who has found mild cases of chytrid fungus but no mass deaths in southern cricket frogs, "it's not a surprise that you get a disease."

What's more, many researchers, noting that there are correlations with environmental factors, still favor this kind of explanation. "It's way too early to rule out these other factors, such as pollutants and UV light," says Andrew Blaustein, an ecologist at Oregon State University in Corvallis. Veterinary researchers such as Nichols add that most fungal infections in amphibians are opportunistic, moving in when animals are already stressed or injured from other sources. And in the United States, there's no clear pattern yet of deaths spreading out from one locality, as in Australia.

Wake also questions whether one fungus could kill so many different kinds of amphibians—frogs and toads in 19 families have died from it, and it has infected salamanders too. "I don't know of any other pathogen that kills like that; for example, one that kills all mammals," he says. In his view the genetics are too preliminary to be sure that the chytrid is the same species and strain in Australia and the Americas. Until that has been shown, he says, "any suggestion of an epidemic [is] irresponsible." Even Nichols, who proved that the chytrid can fatally infect frogs, says to "count me among the skeptics who wonder

CREDITS: (TOP TO BOTTOM) DON NICHOLS/SMITHSONIAN INSTITUTON VETERINARY HOSPITAL; MICHAEL J. SREDL

what role the chytrids are playing" in the wild. He and others question whether something hasn't changed in the frogs' environment to weaken their resistance and promote the chytrid.

While U.S. researchers argue about the chytrid's role, in Australia, Speare, Aplin, and others are waging a campaign to try to stop it. They're tracking the fungus's spread, identifying susceptible species, and planning captive breeding programs. And even skeptical U.S. researchers are urging field precautions, in case herpetologists themselves are spreading the fungus via wet boots or collecting gear. Chytrid specialist Longcore, who named the new genus, notes that she brought a non–disease-causing type of chytrid from Puerto Rico home to Maine in the wet mud on her boots.

Despite all such efforts, Speare and others fear that in Australia, the disease "will be spread like the plague" through new populations. At least, Aplin says, this time scientists will be able to watch one of these sudden declines in action, rather than discovering it after it's all over: "We've caught it this time close to the beginning." And that may provide answers to the many questions that still surround this strange frog killer.

–VIRGINIA MORELL

FROG DECLINES
▶ DEFORMITIES

A Trematode Parasite Causes Some Frog Deformities

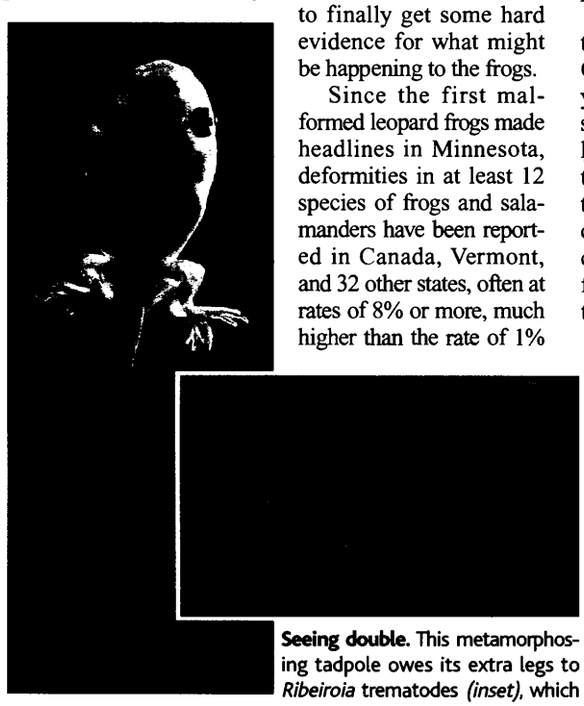
The cysts formed by the trematode lead to abnormal limb development in California frogs; whether trematodes cause the deformities elsewhere remains to be seen

It was a disturbing sign that something might be going terribly wrong in the environment: Frogs with extra legs, missing limbs, and twisted jaws were popping up in ponds across the country. First spotted by schoolchildren in Minnesota in 1995, the famous malformed frogs, together with reports of declining frog populations worldwide, sparked concerns that the animals might be falling victim to some type of environmental degradation—a change that might even threaten human populations. The discoveries touched off a million-dollar-plus hunt to find the culprit, whether natural or humanmade. Two reports published in this issue now point to a natural cause for at least some of the frog abnormalities.

On page 802, a team led by a recent Stanford graduate describes results indicating that infection by a trematode, a kind of parasitic flatworm, is at fault. The researchers based this conclusion on experiments in which they showed that they could exactly duplicate the kinds of limb abnormalities and other deformities seen in California by infecting tadpoles with the trematode, which goes by the genus name *Ribeiroia*. Some experts say that this work, together with a second study reported on page 800 that may exonerate certain chemicals suspected of causing the abnormalities, has now elevated parasites to the top of the list of possible causes for the frog deformities across the country. "This is *the* best experimental evidence showing a cause for the limb deformities in amphibians," says Andrew Blaustein, an ecologist at Oregon State University in Corvallis, who has studied whether ultraviolet light could explain the deformities.

Others caution, however, that *Ribeiroia* infections may not explain the different patterns of frog deformities seen outside of California, especially in the Midwest. "I do not believe that there's a single cause" for the deformities, says herpetologist Mike Lannoo of Indiana University School of Medicine in Muncie. Still, many experts are saying that after several years of frustration it's a relief to finally get some hard evidence for what might be happening to the frogs.

Since the first malformed leopard frogs made headlines in Minnesota, deformities in at least 12 species of frogs and salamanders have been reported in Canada, Vermont, and 32 other states, often at rates of 8% or more, much higher than the rate of 1% or less expected in healthy populations. Investigators have pursued three main theories about what might be causing the problems: chemicals such as pesticides, increased ultraviolet light because of ozone destruction, or parasites (*Science*, 19 December 1997, p. 2051).



Seeing double. This metamorphosing tadpole owes its extra legs to *Ribeiroia* trematodes *(inset)*, which are about 500 micrometers long.

CREDITS: (LEFT TO RIGHT) STEVEN HOLT, STOCKPIX.COM; S. SESSIONS/HARTWICK COLLEGE

Despite the flurry of activity, however, no lab had grown a batch of frogs under environmentally relevant conditions and produced the same deformities seen in wild specimens of the same species—until now. Pieter Johnson began this project 2 years ago for his undergraduate thesis at Stanford, with ecologist Paul Ehrlich as his adviser. Johnson investigated some ponds about 45 minutes south of Palo Alto where up to 40% of emerging Pacific treefrogs had deformities, mostly extra, partial, or missing hindlegs. The water tested free of chemical pollutants, but he noticed that the ponds with deformed frogs always had planorbid snails, a first host for *Ribeiroia* trematodes. "That was a pretty substantial clue" that trematodes might explain the deformities, Johnson says.

That idea fit with a proposal developmental biologist Stanley Sessions of Hartwick College in Oneonta, New York, had made years earlier. In work published in 1990, Sessions had shown that he could induce extra legs in salamanders by implanting beads in their developing limbs, presumably because the beads move cells around. Noting that the cysts formed in infected hosts by trematodes could exert the same kind of mechanical forces as the beads, Sessions suggested that the worms could also cause limb deformities.

By the time he graduated last June, Johnson had dissected hundreds of frogs and found that they did in fact have trematode cysts clustered around their extra limbs. But he hadn't done any experiments exposing tadpoles to the parasites. "I couldn't let go that close" to a solution, he says. So he teamed up with two friends, Kevin Lunde and Euan Ritchie. They all spent the summer "working pretty intensely," Johnson recalls, often from 10 p.m. to dawn so they could catch the parasitic worms when they emerged from the snails and use them to infect the frogs.

After several false starts, the team began infecting tadpoles with *Ribeiroia* and watching them develop into adults. The results were "almost painfully textbook," Johnson says. Higher doses of the trematode produced more deformities, and the mix of multiple