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"Science affects the way we think together."
Lewis Thomas

Sleuthing Out a Silent Scourge for Amphibians

William P. Leonard

*This frog (*Rana cascadae*) is one of many amphibian species worldwide threatened by the chytrid fungus,* Batrachochytrium dendrobatidis *(*Bd*).*

IN SUMMARY

The amphibian chytrid fungus, Batrachochytrium dendrobatidis *(*Bd*), causes the infectious disease chytridiomycosis, which has triggered massive die-offs and extinctions of amphibians around the world. The disease, identified in 1998, is a significant contributor to the global amphibian biodiversity crisis, and no clear means of arresting its spread has been found.*

Conservationists, scientists, and wildlife managers are grappling with understanding the extent and severity of chytrid disease and its ramifications on species and ecosystems. Enlisting collaborators around the world, Dede Olson, with the Pacific Northwest Research Station, and her colleagues initiated a global surveillance project in the form of a website-based database, displayed on publicly accessible maps that show the incidence of Bd *and the affected species.*

The first comprehensive report on the collected data revealed that patterns of infection differed among different species and sites. However, it was evident that biodiversity within amphibian communities and climate factors play significant roles in Bd *occurrence. These and other findings have inspired a barrage of new studies and the project website has grown into an international clearinghouse for science and management strategies pertaining to imperiled amphibians. The project is also fostering a novel model for networking and partnerships to produce and share results more rapidly and on broader scales, which could ultimately benefit many different fields.*

"Why should we care? What difference does it make if some species are extinguished, if even half of all the species on earth disappear? Let me count the ways."
—E.O. Wilson, *The Diversity of Life*

Since the 1980s, researchers around the world have noticed something strange and disturbing: amphibians are vanishing. From the Costa Rican cloud forests, to New Zealand and Australia, to Spain, populations of frogs, toads, and salamanders are winking out. At a 1990 world conference of herpetologists, a consensus emerged that the problem was indeed global, affecting many species in a wide variety of habitats. The situation is especially troubling because frogs and their kin—which keep intimate contact with their natural surroundings—are gauges of the environment's health.

In the wake of the conference, an International Union on the Conservation of Nature (IUCN) task force compiled a list of possible causes of these population crashes. Habitat destruction and fragmentation topped the list. Studies began to address the effects of other factors: pesticides, pollution, elevated ultraviolet radiation (resulting from stratospheric ozone loss), invasive species, and climate change. The findings were mixed, varying with different species and places. But in many cases, the steep population declines were an enigma, and direct causal agents remained elusive.

Then, in 1998, following rapid, massive die-offs of frogs in Australia and Central America, researchers detected a previously unknown organism in the deceased: the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). This fungus, which causes the disease chytridiomycosis, was soon linked to

amphibian mortalities. Although the identification of *Bd* was a welcome breakthrough, it also led to many more questions. Where did the fungus come from? Why was it surging in so many far-flung locations? What species are susceptible to the disease? How was the fungus transmitted, and what could humans do to help curb its spread?

The IUCN's first Global Amphibian Assessment in 2004 reported that roughly one third of the 6300-plus known amphibian species were globally threatened. Six years later, amphibians comprised nearly one quarter of all animal species listed as "critically endangered." The sense of crisis was building, but the extent of *Bd*'s culpability was unclear.

Fortunately, global cooperation to address the phenomenon has been revving up since 2007, when Dede Olson, a research ecologist with the U.S. Forest Service Pacific Northwest Research Station, launched an effort to build a global *Bd* map. Because *Bd* is a threat to amphibians in so many regions, the project quickly gained international collaborators. Those joint efforts have produced a website-based tool to collect *Bd* data and depict it on maps, available to any interested party.

Today, research partnerships are blossoming, and scientists and resource managers are forging closer links. The *Bd*-maps.net website has morphed into an interactive global forum for the exchange of all kinds of information on the fungus, affected species, and strategies for restoring and supporting amphibian populations through environmental engineering. The growing body of work has come to represent a new paradigm for fostering and accelerating science and science-management links on broad geographic scales. The gains are now rippling out to other areas of scientific inquiry, with direct application to wildlife research on emerging infectious diseases.

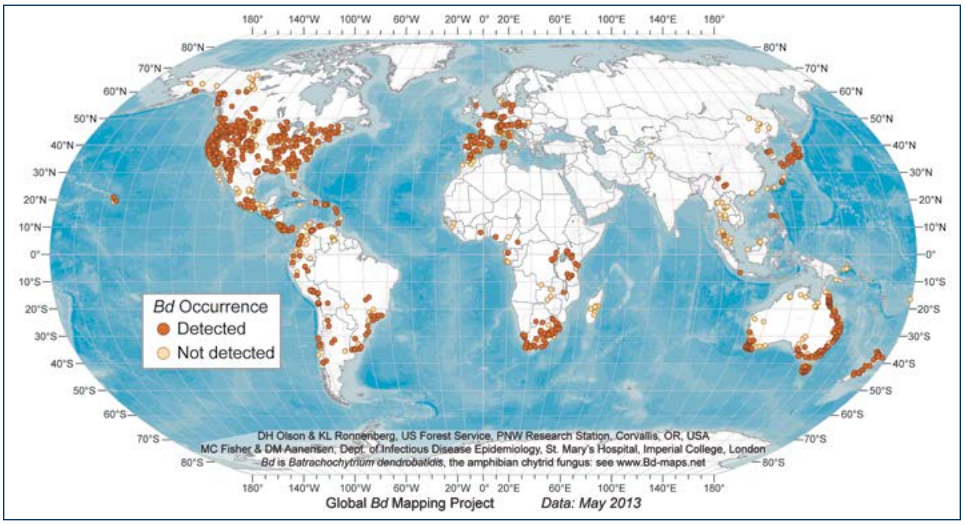
KEY FINDINGS

- The Global *Bd* Mapping Project, begun in 2007, enabled the development of a *Bd* surveillance database that details the occurrences of the fungus and the species affected. As of May 2013, the online maps represented the records of 40,380 animals, sampled at more than 4,000 sites. The fungus was present in about 40 percent of the species tested and in nearly two-thirds of the 82 countries sampled.
- Analysis of the *Bd* data in terms of all affected species at global and USA-only scales revealed patterns of occurrences in association with various environmental and biological factors. In general, *Bd* incidence correlated strongly with the level of species richness within amphibian communities. Climate-related factors, such as the ranges of precipitation and temperature at different sites, were dominant predictors of the odds of *Bd* occurrence, suggesting that climate significantly influences *Bd* occurrence.
- Separate assessments on three families—toads (bufonids), "true" frogs (ranids), and tree frogs (hylids)—showed wide-ranging differences in patterns of infection. This likely reflects environmental traits specific to each group.
- The fungus's overall spatial distribution varies greatly, and it is apparently still spreading rapidly into new geographic areas

FIRST RESULTS

Following 6 years of data collection and analysis, Olson and colleagues recently unveiled the first scientific "big picture" on *Bd*. As of May 2013, the online maps—which allow viewers to visualize the data globally and by country—represented the records of 40,380 animals, belonging to 1,252 species and sampled at more than 4,000 sites. "Thus far, the fungus has showed up in about 42 percent of the species tested and in nearly two-thirds of the 82 countries sampled," Olson reports.

Bd's unusually wide host range is one of its most worrisome aspects. "We know of few other wildlife diseases that readily spread among so many taxa within a vertebrate class," she notes. Another complication is that untold numbers of *Bd*-infected animals harbor the fungus without exhibiting disease symptoms, such as extreme lethargy, skin lesions and shedding, and hampered respiration, which lead to death.



Researchers have tested amphibians for Bd *at more than 4,000 sites around the world. The series of online maps at www.Bd-maps.net draws on information from 40,380 animals, belonging to 1,252 species.*

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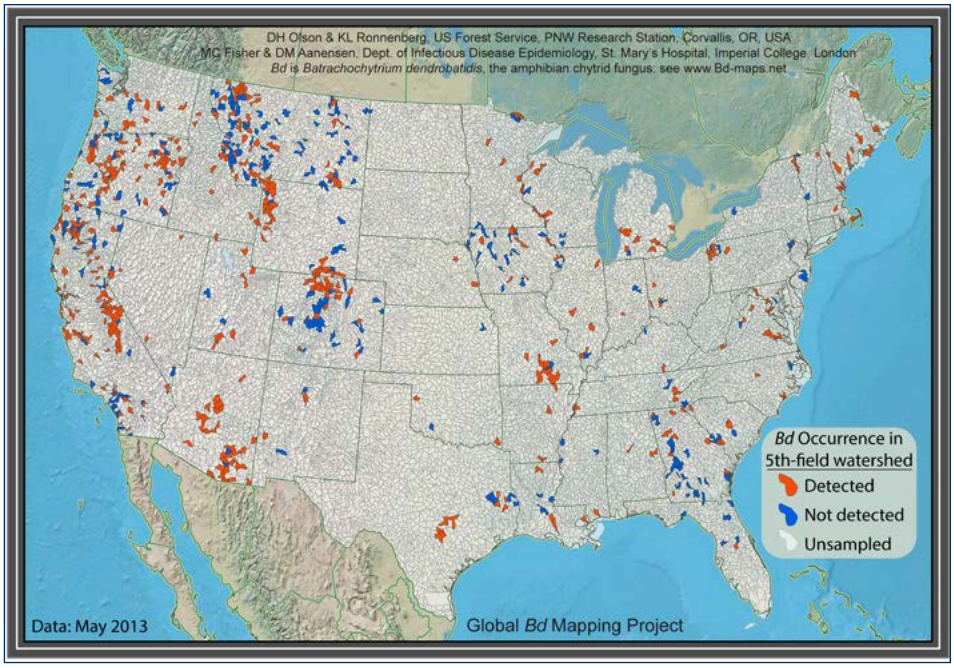
In interpreting the maps, it's clear that the intensity of surveys for *Bd* varies greatly around the world, Olson says. "Some regions—for example, Europe, the United States, and Central America—have many records in the global database, while others, particularly Africa and Asia, have comparatively few. Half of all the compiled sites are located in the United States, where only a handful of species have proved to be susceptible to chytridiomycosis symptoms so far." Also, many amphibian taxonomic groups are not well sampled; researchers are collaborating to close these data gaps, to more fully understand the potential disease threat at more comprehensive geographic and taxonomic scales.

Olson and her colleagues explored environmental and biological factors that could help explain the existence of *Bd* at global and national (USA) scales. To do this, they developed models of *Bd* occurrence that included variables such as temperature and precipitation ranges, landscape features (biome classifications), species diversity within amphibian communities, and previous episodes of unexplained amphibian declines. Their global model covered all *Bd*-affected species. Additional models focused on three amphibian families (separately) at global and USA scales. The three families analyzed were those with the most information in the *Bd* database: ranids ("true" frogs), which generally require water bodies for breeding; hylids ("tree" frogs), which may or may not climb trees, but are generally less closely associated with water, instead seeking out moisture on land for their needs; and bufonids (toads), which, being dry-skinned, thrive in terrestrial habitats.



Sites across the continental United States where Bd *has been found.*

Hannah Anderson

Doctoral student Tara Chestnut swabs a frog in Alaska and then releases it unharmed. The swab will be taken to a laboratory where it will be tested for the DNA of the amphibian chytrid fungus.

One intriguing insight that emerged from the modeling was a strong correlation between the incidence of *Bd* and the species richness of amphibian communities. "Presumably this is because a higher number of species potentially carrying the fungus boosts the probability of it spreading," Olson explains. "And, it suggests that diversity may affect disease occurrence and transmission rates." Furthermore, the correlation between *Bd* occurrence and species richness at a site increased when sites with unexplained declines were separated out from the pool of data, indirectly supporting disease as a cause of many past die-offs of amphibians where other plausible causes were lacking.

Another stand-out result was that climate-related factors were dominant predictors of the odds of *Bd* occurrence. This was a big surprise, Olson says. "For example, we knew that as an aquatic organism *Bd* thrives in relatively cold water (about 59 °F). But now the data have shown that it's much less likely to be present in areas with wide swings in air temperatures." Laboratory studies show *Bd* can exist at temperatures between 39 and 77 °F, and it stops growing at about 82 °F. Areas with wide ranges in temperatures—for instance, from below freezing to summer heat waves—may prove inhospitable to the fungus, or may have amphibian inhabitants that can more readily shed the disease.

All of this suggests that climate significantly influences *Bd* occurrence. This understanding is guiding researchers in their investigations to better determine where the fungus is currently prevalent and where it's likely to show up based on future climate predictions.

Not surprisingly, the modeling results for individual amphibian families—not all of which are native to all regions of the world—varied considerably. For example, the odds of detecting *Bd* in toads (bufonids) were greatest in boreal and temperate coniferous forests, and increased steeply (by 54 percent) with every 3,300-foot elevation gain. For tree frogs (hylids), the highest incidences of *Bd* infection occurred in montane grasslands and shrublands and in dry broadleaf tropical forests, increasing in tandem with average annual temperature, but decreasing as the average temperature range expanded. As for true frogs (ranids), the odds of infection rose with each degree of average temperature and decreased substantially with every 3,300-foot gain in elevation. The fungus has been most prevalent in ranids of tropical and subtropical grasslands, deserts, and dry shrublands.

"The wide-ranging differences in patterns of infection between the families we examined likely reflects environmental traits specific to each group," Olson explains. "Overall we see that *Bd*'s spatial distribution varies greatly. And, it appears that it's still spreading rapidly into new geographic areas, so it hasn't yet reached a global equilibrium," she says.

USING THE MAPS TO EXPLORE NEW SCIENTIFIC TERRAIN

The growing body of knowledge about *Bd* is prompting new questions and spurring a flurry of research at finer scales of inquiry.

One critical topic revolves around how and why species differ in their susceptibility to *Bd*. "We have identified patterns such as over- and under-infected families. And we have seen in laboratory tests that the progress of infection in animals of different species inoculated with the fungus varies over time, as do the mortality rates," Olson says. "But what makes some animals resistant to infection and developing chytridiomycosis, and others not?"

One hypothesis is that resistant animals are equipped with immunological or physiological traits that protect them from infection. Following a recent chytrid mortality event, surviving frogs were found to carry some 500 types of microbes on their skin, prompting researchers to begin scrutinizing this microbial community to learn if one or more of these organisms might help ward off *Bd* infection. If such an agent is identified, it might be possible to develop a way to inoculate vulnerable groups, Olson suggests. Synergistic effects of multiple stressors may also be involved, for example, if an environmental change is affecting the immune response of amphibians, reducing their ability to ward off disease.

As amphibians contend with the new disease environment brought on by *Bd*, natural selection dictates that species will adapt or fade away. "Our findings are stimulating interesting evolutionary questions," Olson says. "For instance, given that the fungus is aquatic, might amphibians become more 'reptilian' in their behavior or physiology to elude infection? Could social and breeding systems be shifting in ways that could curb frog-to-frog *Bd* transmission? Will areas with large temperature ranges become amphibian refugia, where species will prevail disease-free?" Such changes may already be happening. For instance, there's evidence that some tree frogs are modifying their habit of skin-shedding, increasing its frequency and even inducing fevers that dry out the fungus.

The fungus is also evolving. Many genetic strains of *Bd* have now been identified. These strains differ in virulence (potentially influencing which species succumb to infection), and their potency wanes over time, as strains are repeatedly cultured in laboratory settings. "Natural selection is happening; it's evolutionary biology on the fast track," Olson says. "Such discoveries can help us better understand the intricacies of infectious disease in animals, perhaps including humans."

Elke Wind

Researchers found that for tree frogs, incidences of Bd *infection increased with average annual temperatures, but decreased as the range of average temperatures expanded.*

Bd TRANSMISSION AND THE HUMAN CONNECTION

Bd is now officially considered an emerging infectious disease and an aquatic invasive species, but the question of how it's spreading is still a mystery. "There is a simple test for identifying fungal spores in water, and *Bd* has been found in surface waters as far north as Alaska," says Olson. Birds, insects, and other animals besides amphibians might pick up water with *Bd* and transport it. "And as people move either animals or water, we could be spreading the fungus," Olson says.

The origins of chytrid point to Africa or Asia, where *Bd* has been detected in archived frog specimens dating back to the 1800s. The fungus is common among some frog species in several African countries, but population declines there are poorly documented, and so far no chytrid-associated die-offs have been reported. However, one common carrier of *Bd*, the African clawed frog, may have played a seminal role in its global dissemination.

In 1934 a new way of detecting human pregnancies using African clawed frogs came on the market, triggering the capture and worldwide export of enormous numbers of the species from southern Africa—a practice that continued into the 1970s. "African clawed frogs, which are largely resistant to chytrid, also proved useful in other biomedical applications, and accidental and deliberate releases into the wild have almost surely exacerbated the spread of the fungus," Olson says.

Another chytrid vector abetted by humans is the American bullfrog. Native to the eastern United States, the bullfrog is also virtually immune to chytrid disease. It has long been farmed for food markets, and thanks to escapes and intentional releases, the bullfrog has become an aggressive invasive species in many states. "The effects of globalization and human activities that involve transporting known and potential hosts of *Bd* have unwittingly played a huge role in facilitating the spread of the fungus," Olson concludes. "On the hopeful side, understanding how this has happened can inform our efforts to reduce the human end of the equation."

Bd-maps are critical to this effort. The maps, which are continually updated, allow conservation personnel, land managers, and government agencies to assess infection risk at regional and local levels and follow up with steps to forestall the spread of chytrid. For example, the U.S. Fish and Wildlife Service and the World Wildlife Disease Organization have both used project data in addressing amphibian trade issues while evaluating trade policies in this country and internationally.

The map data are also aiding development of protective measures recommended for field personnel, such as disinfecting instruments and equipment that's used in multiple locations. And, Olson and her team have devised a watershed-scale map of the United States that depicts *Bd*-contaminated water sources, which can be used in evaluating the risk of disease transmission where water-diversion projects, wildfire management, and other activities might involve the cross-watershed transfer of water. For example, Olson says, "Fighting wildfires often depends on making water draws from nearby water sources. If fire personnel know which sources harbor the fungus, they may be able to choose an alternative, uncontaminated source."

The significant progress made in the field of *Bd* science since 2007 reflects wider changes in how research is being conducted in our technologically connected world, Olson notes. "In a sense, there's a renaissance of science happening, and the effects are similar to social networking on the Internet. The science process is accelerating and pushing our advances faster and farther."

Despite these gains, as yet there's no panacea in sight for curbing the spread of the disease. Conservation priorities are often constrained by limited funding and capacity. Faced with limited resources and capacities, many wildlife managers charged with overseeing amphibians have become necessarily pragmatic, turning their efforts to sustaining the survivors. "While the ideal is to forestall threats, without a sword to defend against chytridiomycosis in our armory, we can celebrate the life that remains," Olson concludes.

Dede Olson

The U.S. Fish and Wildlife Service and World Wildlife Disease Organization have used data from the Bd-*mapping project to evaluate amphibian trade policies in the United States and internationally.*

"Conservation is a state of harmony between man and land."
—Aldo Leopold

FOR FURTHER READING

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LAND MANAGEMENT IMPLICATIONS

- *Bd*-maps.net is being accessed by land, species, and disease managers and other decisionmakers to assess chytrid risk at local, regional, and global scales and develop policies to forestall the spread of disease. The *Bd* data has been applied to issues such as amphibian trade regulation, disinfection protocols for field personnel, and human-mediated water transfers.
- As the first major effort to map a global wildlife disease, based on the cooperation of an entire world biological community, the Global *Bd* Mapping Project serves as a model for other such efforts in confronting emerging infectious diseases and fostering widespread social networking among scientists and managers. The project's novel approach of displaying a global surveillance database online in an interactive format is being applied to create the Ranavirus Reporting System.
- The gains in understanding chytrid disease dynamics, associated environmental factors, and susceptibility among taxonomic groups is prompting new avenues of scientific study and potential management strategies for supporting vulnerable amphibian populations.

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