Credit: Dexter Mardis

▲ **Crusty eyes and deformed scales — signs of snake fungal disease — appear on a speckled kingsnake that Dexter Mardis collected on a private ranch in eastern Kansas in April 2016. The discovery of the disease here marked a new westernmost point for snake fungal disease, which has been wiping out vulnerable snake populations across the country.**

Out of Hand

BIOLOGISTS GRAPPLE WITH FUNGAL DISEASES

By David Frey

Dexter Mardis was about to quit his search. He had spent the morning scrambling through rough-hewn creek valleys that cut through wild south-eastern Kansas tallgrass prairie, on the lookout for snakes. He hiked among flat limestone bluffs that rose so evenly above the ravines they looked like castle walls, turning over rocks and peering underneath as he walked. He knew he would find plenty of snakes fresh from hibernation on this cool morning in April 2016, but he wasn't prepared for what he was about to uncover.

Two years earlier,

Wichita State University was granted access to the 4,700-acre private Youngmeyer Ranch for research. Mardis, the university's biological field station manager and president of the Kansas Herpetological Society, wanted to catalog the snakes that called it home. He gathered together a group of 55 herpers and, wearing light jackets, they set off to photograph what they found.

Mardis flipped over a rock that looked promising and was rewarded with a Great Plains rat snake (*Pantherophis emoryi*) that lay coiled underneath. Brown-patched, about 2 ½ feet long, it was a common snake in Kansas. But this one was different. Thick, scabby blisters covered its head. Its lips were so crusted, they looked like they were rotting. Mardis knew snakes sometimes emerge from hibernation with sores, especially after damp winters. The previous winter had been dry, though, and this snake seemed to be in very bad shape.

He had heard about snake fungal disease, a deadly disease wreaking havoc in snake populations across the eastern United States and threatening some species with extinction. About 2 ½ years earlier, he had lectured a high school group about it when they came across a North American racer (*Coluber constrictor*) with a head so blistered its mouth wouldn't close. Those were the kinds of symptoms that East Coast biologists were on the lookout for, he told the students, but he dismissed it. Snake fungal disease hadn't been seen anywhere near Kansas.

But that racer "didn't ever totally leave my mind," Mardis said, and after uncovering this strange-looking rat snake, "it popped right back up again."

Mardis scooped up the rat snake so he could examine it later. By the end of the day, he had collected three speckled kingsnakes (*Lampropeltis holbrooki*) and another rat snake, all with similar lesions. The volunteers snapped photos of even more. When Mardis' specimens were tested, all five were positive for snake fungal disease. The find made Kansas the 10th state in the country where the disease has been documented and its westernmost point in the U. S. so far.

"I think [snake fungal disease] could potentially pop up anywhere in North America," said Jeffrey Lorch, microbiologist with the U.S. Geological Survey's National Wildlife Health Center in Madison, Wis., a leading snake fungal disease researcher who tested these snakes.

The disease that was killing snakes is part of a wave of fungal diseases taking their toll on several groups of wildlife in the U.S. and around the world over the past two decades. But what's been especially worrisome for wildlife managers is that these diseases are decimating populations, hitting some endangered and threatened species particularly hard. And so far, there's little to suggest the worst is over.

'Pandora's Box'

"Unwittingly, humanity has opened a Pandora's box of emerging fungal infections that are now causing a tsunami of biodiversity loss in frogs, bats, snakes and other wildlife species," Matthew

Credit: Brad Glorioso, USGS National Wetlands Research Center

▲ **Biologists fear a fungal disease killing off salamanders in Europe would have devastating effects if it reached North America, threatening species like the marbled salamander (*Ambystoma opacum*).**

Credit: D. Edmonds, USGS

▲ **A Sierra Nevada yellow-legged frog, one of the North American species most threatened by a deadly chytrid fungus, sits alongside a lake in Yosemite National Park.**

Credit: USGS

▲ **The white filaments of the fungus that causes white-nose syndrome in bats appears on items, possibly scat, in a New York mine.**

C. Fisher, professor of fungal Disease Epidemiology at Imperial College London, wrote in Britain's journal *Philosophical Transactions of the Royal Society B*, which devoted an entire issue to the worldwide rise of fungal diseases last December (Fisher et al, 2016).

As wildlife professionals know, a chytrid fungus (*Batrachochytrium dendrobatidis*, or Bd) has ravaged global amphibian populations, threatening thousands of species — particularly frogs and toads — with extinction, including the boreal toad (*Anaxyrus boreas boreas*) and the mountain yellow-legged frog (*Rana muscosa*). A related fungus (*Batrachochytrium salamandrivorans,* or Bsal,) has hit salamanders in Europe recently, worrying North American wildlife managers that if it hit this continent, it could devastate the greatest diversity of salamanders found anywhere in the world. White-nose syndrome — a fungal disease caused by *Pseudogymnoascus destructans* that invades the skin of hibernating bats and disrupts their hibernation physiology — has killed off more than 5 million bats across much of the U.S. and Canada, in some cases wiping out entire cave populations and further impacting some species that were already facing extinction.

These diseases have become so widespread and deadly, Fisher argued, partly because of global trade, which has allowed pathogens to spread around the world more easily, and partly because of environmental degradation that may make some species of wildlife more vulnerable to disease.

"In both animals and plants," he wrote in the journal *Nature* (Fisher et al, 2012), "an unprecedented number of fungal and fungal-like diseases have recently caused some of the most severe die-offs and extinctions ever witnessed in wild species."

Fungal diseases can be particularly hard to combat, biologists say, because unlike viruses, the fungi don't rely on their hosts to survive. Their spores can survive for years and sometimes decades. "That's what makes these fungal pathogens of wildlife so alarming," said David Blehert, branch chief of the USGS's Wildlife Disease Diagnostic Laboratories in Madison, Wis. He says a fungal pathogen can emerge, wipe out a population and retreat to the ground again until another host arrives.

Disappearing frogs

The disappearance of frogs first caught biologists' attention in the 1990s. From the southern day frog in Australia (*Taudactylus diurnus*) to the northern Darwin's frog in Chile (*Rhinoderma rufum)*, frog populations across the globe were dwindling or vanishing altogether, and scientists didn't know why.

"People started to notice that frogs were disappearing in the places they were working," said TWS member Evan Grant, a USGS wildlife biologist at the Patuxent Wildlife Research Center in Turners Falls, Mass. "From that point on, they started looking in earnest to see what was causing those declines."

In 1998, researchers believed they had found the cause. They placed the blame on the amphibian

Credit: USGS

▲ **USGS biologist Evan Grant processes field data during a survey of amphibians. Biologists "are trying to figure out the most important research to be doing," Grant said, "to try to provide managers with the information they need to make more informed and better decisions."**

Credit: Chris Brown, USGS National Wetlands Research Center

▲ **A mountain yellow-legged frog perches on a rock. The species is among those in North America most affected by a fungal disease attacking frogs and toads around the world. Biologists call it the greatest extinction event since the Pleistocene.**

chytrid fungus known for short as Bd. The fungus was causing the animals' skin to thicken and disfigure – a condition that kills amphibians, which absorb water and electrolytes through their skin. The disease, chytridiomycosis, was taxing their biological systems until their hearts gave out.

A dramatic die-off of amphibians ensued. Biologists say worldwide amphibian extinctions are 105 times higher than the background rate, making this the greatest extinction event since the Pleistocene extinctions wiped out mammoths, mastodons and saber-tooth cats 11,000 years ago. The International Union for Conservation of Nature estimates 41 percent of the 6,771 identified amphibian species are extinct or threatened with extinction, due in large part to the chytrid fungus. A recent large-scale analysis of data from across the U. S. finds that populations continue to disappear at an average rate of 3.8 percent each year (Grant et al 2016). In the U. S., Bd is thought to be mostly affecting montane species, while globally, neotropical frogs are taking the biggest hit.

Some research indicates the fungus may have become so deadly in part due to the pet trade, which allowed what might have once been a local outbreak of a fungal disease to reach amphibian populations in parts of the globe where the disease had never been seen (Vredenburg et al. 2013).

Some biologists believe the rise of this disease may be linked to climate change. In his 2006 study published in *Nature*, biologist Alan Pounds concluded that warming Costa Rican cloud forests allowed fungi like chytrid to thrive, killing off species like the golden toad (*Incilius periglenes)* and Monteverde harlequin frog (*Atelopus varius*) (Pounds et al 2006).

"With climate change promoting infectious disease and eroding biodiversity, the urgency of reducing greenhouse-gas concentrations is now undeniable," he wrote.

Spanish researchers also found a link between the spread of chytridiomycosis and rising temperatures (Bosch et al 2006).

A related fungus, *Batrachochytrium salamandrivorans,* or Bsal, has devastated salamanders in Europe. Scientists have found no cases in the U.S., and they're hoping to keep it that way. An outbreak of Bsal here, they worry, could be devastating.

"The Appalachians are the center of biodiversity for salamanders in the world," Grant said.

Bats wiped out

Researchers soon discovered it wasn't just amphibians that were being wiped out by fungal diseases.

Credit: USGS

Biologists and technicians prepare to enter a New York mine containing bats with white-nose syndrome. First discovered in New York, the disease has wiped out millions of bats in North America.

In March 2007, a team of New York Department of Environmental Conservation biologists were conducting a count of endangered Indiana bats when they entered Hailes Cave, a major hibernation site for bats in John Boyd Thacher State Park, about 15 miles southwest of Albany. The team stepped into the cave and found thousands of dead bats and bat parts littering the cave floor. Two years earlier, they had tallied about 15,000 bats hibernating in the cave. This time, the number was cut in half, and among the living, about half had a strange white fungus around their muzzles. The bats seemed unusually unresponsive, they noticed, and they were clustered around the cave entrance. Reports came in of similar bat deaths in four caves close by. Then a caver came forward with photos he shot a year earlier in nearby Howes Cave, which is connected underground to a commercial cave popular with tourists. In a wild and less-traveled section of the cave, he counted as many as 18 dead bats. His photos showed living bats marked with the telltale white fungus.

Biologists were dumbfounded. They had never seen a disease like this. They called it white-nose syndrome for obvious reasons.

"It was absolutely a blank slate," said TWS member Jeremy Coleman, national white-nose syndrome coordinator for the U.S. Fish and Wildlife Service. "This was a fungus that was not even known to science. It was not named. Nobody had ever seen it before. Since that time we've learned an incredible amount about the fungus, and about the disease."

Researchers determined these bats had died of a disease caused by the fungus *Pseudogymnoascus destructans*. Scientists uncovered the fungus in caves throughout Europe and Asia, but it was not known to be nearly as deadly as it became when it mysteriously appeared in North America. That observation has left researchers to wonder how it got to this continent.

Maybe an infected bat arrived on a shipping container, Coleman said, but then, how did that bat find its way into Howes Cave? More likely it came on a cave visitor's boot, he said, although that idea has been controversial among cavers.

"Most likely some form of human activity inadvertently brought it over," said the USGS's Blehert. "Human activity is recognized as the largest driver of emerging infectious diseases worldwide."

White-nose syndrome causes bats to burst into activity during hibernation when they should be resting, burning precious energy and depleting the fat reserves they need to make it through the winter. Affected bats can be seen flying outside during the day and clustering near the entrances of the caves and mines where they should be saving their energy through the winter. In some hibernacula, white-nose syndrome has killed off 90 to 100 percent of the bats.

Credit: USGS

USGS biologists Doug Berndt, left, and David Blehert, conduct necropsies on bats at the USGS National Wildlife Health Center in Madison, Wis.

"From what we're seeing currently, unless we come up with management or treatment options, I think we're looking at the potential extinction of some species," Coleman said.

Since it was discovered, white-nose syndrome has killed off 5.7 million bats in 29 states and five Canadian provinces, mostly in the East, including the little brown bat (*Myotis lucifugus*), the tricolored bat (*Perimyotis subflavus*) and the threatened Northern long-eared bat (*Myotis septentrionalis*). In the winter of 2015-2016, it seemed to leap across the country and appeared in little brown bats in Washington state, leaving researchers to speculate again that humans somehow carried the disease from one side of the continent to the other.

"Oceans and mountain ranges used to limit our ability to move about," Blehert says. "They really don't anymore."

'Something massively wrong' with snakes

Snakes became the next victims of fungal disease.

In 2009, veterinarian Matt Allender was chatting with graduate students at the University of Illinois as they prepared to collect snake specimens. One of them recalled "some weird snakes" they found the previous year, snakes so deformed the students assumed they had been hit by cars.

"I hope we don't have them again," he remembered the student saying.

Allender asked to see these mysterious eastern massasauga rattlesnakes (*Sistrurus catenatus)*, brown-spotted vipers found throughout the Midwest. The students took the jars of ethanol where the snakes had been stored and opened them in the lab. Allender thought it would be a good chance to teach about wildlife mortality, but when he saw them, he realized these snakes weren't hit by any car.

"It was pretty apparent from the moment I put my eyes on them that there was something massively wrong," said Allender, assistant professor of veterinary clinical medicine and director of the Wildlife Epidemiology Lab at Illinois' College of Veterinary Medicine. "The heads were completely disfigured. There were massive amounts of swelling and crust and pustules and lesions."

Credit: Kyle Gabriel

Students at Georgia State University conduct a survey at the Atlanta Zoo for the fungus that causes snake fungal disease.

Growing up catching snakes in Illinois, Allender was used to seeing snakes with skin damage. But as he examined these snakes, he began to realize something was affecting these snakes unlike anything he had seen before. He would soon discover that what was wrong with these snakes was killing snakes across much of the U.S. These were the first identified free-ranging wild snakes with a disease that has come to be known as snake fungal disease, a condition devastating snake populations across the country – particularly some of the most fragile and threatened.

Research suggests the disease causes snakes to molt more often and exhibit strange behaviors like anorexia and lingering in open areas, which make them vulnerable to predators and starvation (Lorch et al. 2016).

"This was something that could potentially affect conservation efforts and would definitely impact the management of snakes," Allender said.

When he first saw it, Allender believed it was another chytrid disease, like the ones wiping out amphibians. Instead, researchers discovered it was a separate fungus, *Ophidiomyces ophiodiicola*, or Oo.

Unlike the diseases killing bats and amphibians, snake fungal disease appears to be a homegrown disease. Biologists say the pattern of its occurrences doesn't suggest that it's spreading, but that researchers are simply discovering it in new areas as they see it appearing in an array of different snakes, from the common watersnake (*Nerodia sipedon*), to the North American racer (*Coluber constrictor)* to the eastern milksnake (*Lampropeltis triangulum*).

Allender's rattlers weren't the first to have the disease, it turned out. Since he first published his findings about "an unusual mortality event" among eastern massasaugas (Allender et al 2011), it's been traced back to nine dusky pigmy rattlesnakes (*Sistrurus miliarius barbouri*) found with strange lesions at Lake Woodruff National Wildlife refuge in Florida in 1997. Researchers say the disease may have been around decades longer but only became so lethal recently as snakes deal with environmental damage that has made it harder for them to fight it off. It's hit isolated populations particularly hard. In New Hampshire, it wiped out more than half the state's struggling population of timber rattlers (*Crotalus horridus*).

"It's probably due to changes in environmental conditions that facilitate a disease that usually is rather mild to become more prevalent and cause more severe infection," said Lorch, the National Wildlife Health Center microbiologist.

Shrinking habitat may be forcing more snakes to hibernate in the same place, causing snakes to be exposed to greater amounts of the fungus, Lorch said. Habitat fragmentation may be making it harder for populations to rebound. Fire suppression may be allowing trees to grow in areas that were once open, eliminating areas where snakes could once bask, raising their body temperatures high enough to combat the infection.

It may also "be emerging due to climate change," Lorch said. Warmer winters may be allowing the fungus to grow better on snakes while they hibernate. Wetter springs may make the fungus more persistent in the environment and prevent snakes from finding warm, dry places to fend off infection.

"If it's climate change-related," he said, "my concern is it's the tip of the iceberg; that it's the first of a whole bunch of diseases that manifest as a result of host stress."

Can they be stopped?

As these fungal diseases take their toll, biologists have scrambled for ways to check their spread.

In January 2016, the U.S. Fish and Wildlife Service imposed a ban on importing 201 salamander species in an effort to prevent Bsal from entering the country and killing off native salamanders. The ban was imposed as part of an interim rule under the Lacey Act, declaring the species "injurious wildlife."

"The Bsal fungus has the ability to devastate our native salamander populations, and we are doing everything in our power to protect and preserve

Credit: Brad M. Glorioso

Credit: Brad M. Glorioso

▲ **A couple walks a dog near Lake Martin at the Cypress Island Preserve in St. Martin Parish, La. Snakes are commonly found in the area, says biologist Brad Glorioso, who discovered the state's first documented case of snake fungal disease nearby. The underside of a Louisiana pine snake (inset) shows lesions caused by the disease. The species is a candidate for the endangered species list.**

these essential amphibians for future generations," former Service Director Dan Ashe said when the ban was announced.

Researchers have also been seeking ways to treat infected animals and kill the diseases in the wild. Biologists working in Spain found that applying a fungicide to frogs could clear the chytrid fungus from their bodies, but the frogs became infected again when they were reintroduced to ponds. When the ponds were treated with Virkon, a common lab disinfectant, the chytrid seemed to be eliminated without harming the frogs (Bosch et al. 2015).

Biologists have encouraged decontamination protocols to keep cavers — and scientists — from spreading white-nose syndrome from cave to cave. U.S. Forest Service researchers have also made strides in combating the disease. They have found naturally occurring bacteria, yeasts and chemical inhibitors that seem to slow the growth of the fungus and allow individual bats to survive. In 2015, 75 infected bats were released into the Mark Twain Cave Complex in Hannibal, Mo., after they were treated. Scientists placed the bats in a cooler with bacteria that release volatile organic compounds. These compounds slowed the growth of the fungus and allowed the bats to recover.

Treating an entire cave is trickier, though. Researchers worry the bacteria would disrupt a cave's delicate ecosystem if the bacteria were deployed on a large scale. "Most treatments are not likely to be deployed in natural cave environments," Coleman said, "particularly those containing other species of concerns."

Some bat species also seem to be resistant to the disease, meaning they may thrive in caves where other bats have died.

"The good news is we will continue to have bats in North America after white-nose syndrome," Coleman said, "but they may be a different assemblage of species."

Meanwhile, Allender has found bleach and several household cleaners were effective in stopping snake fungal disease, and in a paper he's preparing to publish, he has found antifungal medications delivered using an over-the-counter nebulizer, often used to treat asthma in children, also may help individual snakes overcome the disease.

With good palliative care, a snake may recover, said Brad Glorioso, an ecologist with the Amphibian Research and Monitoring Initiative Team at USGS's Wetland and Aquatic Research Center in Lafayette, La.

In 2015, Glorioso found that state's first confirmed case of snake fungal disease. He's seen dozens more since then, including a Louisiana pine snake *(Pituophis ruthveni)*, which biologists are trying to nurse back to health. The species is a candidate for Endangered Species Act listing.

"It's just another stressor for a snake we're already worried about," he said.

Finding a solution across a habitat has been elusive, though. Tests found an agricultural fungicide didn't kill the fungus, leaving biologists wondering what can be done to help the snakes survive.

"At a population level," Glorioso said, "I don't know if there's much we can do." ■

David Frey is an editor at The Wildlife Society.