Missouri ponds provide clue to killer frog disease

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A green frog, *Rana* (*Lithobates*) *clamitans*, in a pond at Washington University's Tyson Research Center. In Missouri, this frog's tadpoles are often infected with the amphibian chytrid fungus but rarely sickened by it, creating an opportunity to delve into chytrid's place in aquatic ecosystems. Credit: Travis Mohrman/Tyson Research Center

The skin fungus, *Batrachochytrium dendrobatidis* (Bd), also known as amphibian chytrid, first made its presence felt in 1993 when dead and dying frogs began turning up in Queensland, Australia. Since then it has sickened and killed frogs, toads, salamanders and other amphibians worldwide, driving hundreds of species to extinction.

As a postdoctoral researcher Kevin Smith studied Bd in South Africa, home to the African clawed frog, a suspected vector for the fungus. When he took a position at Washington University in St. Louis, where he is now interim director of the Tyson Research Center and adjunct professor of biology, he worked on other problems.

But whenever he visited a pond, he collected tadpoles and checked their mouth parts (often a fungal hot spot) under the microscope, just out of curiosity.

Wading in

So Smith recruited a team of students to study the ecosystems of 29 ponds in east-central Missouri. The team assayed larval amphibians for chytrid, collected physical and chemical data, and identified amphibian, macroinvertebrate and zooplankton species living in the ponds.

"I was half expecting it to be just an absolute mess, that there would be no distinguishing characteristic about ponds that have chytrid or ponds that don't," he said. "But instead, we found that the ponds that had chytrid were consistently more similar to one another than the ponds that didn't have chytrid in many different measures."

"That's a very powerful finding," he said. "The thinking had been that chytrid required keratin, appropriate temperatures and water—and that was it. That's what we were stuck with. Now we know that there must be additional constraints because some ponds that meet these criteria don't harbor the fungus."

A statistical technique for ferreting out causal relationships suggested that this pattern was an indirect effect mediated by the ponds' invertebrate communities.

"They may be alternative hosts," Smith said. "That would be the most parsimonious explanation." But they might also be reservoirs (sites where the fungus can survive when there is no available host).

"The presence or absence of alternative hosts or reservoirs has a huge effect on the dynamics of the disease, and ultimately on the fate of the amphibians it attacks. If there are reservoirs we need to know about them, because otherwise it will be impossible to interrupt the chain of

transmission," he said.

More evidence has since accumulated against the suspect group that fell out of the statistics of the pond study.

One group, looking for a model organism that could be used to study chytrid, showed that it can infect and kill the nematode Caenorhabditis elegans. Another team reported that crayfish are able to transmit chytrid, which infects the lining of their gastrointestinal tracts.

"Focusing only on amphibians to understand chytrid is like focusing only on people to understand Lyme disease," Smith said. "In the case of Lyme disease, we know that mice matter, that deer matter, that oak trees matter. Many different factors lead to there being a lot of Lyme in some cases and not others," Smith said.

Smith hopes that research in areas where chytrid is endemic may be able to help amphibians in areas where it is epidemic. The only alternative so far is the Amphibian Ark, a global effort to maintain threatened amphibians in captivity until they can be "secured in the wild."

A central newt, *Notophthalmus viridescens*, makes its home in a Missouri pond. Newts are a common pond amphibian in Missouri and are often infected with the amphibian chytrid fungus. Credit: Tyson Research Center

He found the fungus in about a third of the ponds whose tadpoles he checked. The obvious question was why only a third? Why didn't it occur in all amphibian populations in a region where it is found?

The amphibians and the fungus have reached an evolutionary truce in Missouri, where the chytrid is endemic rather than epidemic. Because there was no pressure to rescue an amphibian population, Smith had the time and the opportunity to look more broadly and to study the entire pond ecosystem.

Together with then-undergraduate student Alex Strauss, Smith collected physical and chemical data and surveyed the species living in 29 ponds in east-central Missouri. The results of this study are published in the Sept. 25 edition of *PLOS ONE*.

Somewhat to Smith's surprise, it was statistically possible to distinguish infected from non-infected ponds, a finding he likens to being able to predict that influenza will circulate in some cities but not others.

"We don't know exactly what the key factors are but just knowing that not every pond appears to be suitable for chytrid in a given year is a very big step," he said.

The study also suggested that patterns of Bd infection might be an indirect effect of variations in invertebrate communities. What this meant was unclear, since chytrid was thought to be an amphibian specialist.

But while the pond study was underway other researchers announced that crayfish and nematodes can be infected with chytrid, raising the possibility that invertebrates act as alternative hosts or biological reservoirs for the fungus.

"Alternative hosts and reservoirs have been a key missing piece in our understanding of chytrid epidemiology," Smith said. The fungus, like any pathogen, cannot be effectively controlled unless all its hiding places are known.

An ancient fungus

Chytrid, or more properly amphibian chytrid, since there are about 1,000 species of fungus in the class Chytridiomycetes, specializes on keratin, a structural protein found in the skin, hair, nails and similar tissues of vertebrates.

"As far as we know, it doesn't infect any other animal protein," Smith said. "So that's one of the most important restrictions on where it lives."

In amphibians, chytrid infects and damages the skin, which amphibians use to breathe and absorb water. Once the fungus takes hold, it causes a disease called chytridiomycosis, which is usually fatal.

"You can sometimes tell when a frog is infected," Smith said, "by the way it walks. It is slow and spraddles its legs, as though its skin is painful or chafed. When we grabbed frogs like those in South Africa and took samples, they were always heavily infected with the fungus," he said.

Unlike more familiar fungi such as mushrooms, which release spores that drift through the air, chytrids, among the earliest fungi to evolve, are aquatic and release flagellated zoospores that swim through the water.

"Laboratory studies suggest the zoospores can live independently only about a day or so. They're considered to be very fragile," Smith said. "They get expunged from the fungal cell inside the amphibian skin, they swim around for about a day, and if they don't infect something with keratin, they're no longer viable. That's what's generally thought.

"That's why we focused on the aquatic habitat," Smith said. "Animals may be able to move the fungus from one location to another, but it's not just drifting in the air. Our question was: If the aquatic habitat is key, why don't we find chytrid in every aquatic habitat?"

The big picture

As a community and conservation ecologist, Smith suspected the answer couldn't be found by selectively studying the amphibians dying of chytrid. Scientists racing to save amphibian species from extinction have understandably tended to narrow their focus to the pathogen and its victims.

"It's the crisis of amphibians dying and going extinct that makes us focus so narrowly," Smith said.

But Smith has never seen any evidence that chytrid causes mortality in this part of Missouri, although it is one of many factors leading to the decline of hellbenders, a large salamander native to the Ozarks.

Because chytrid is an endemic disease in Missouri, Smith realized he could back up, slow down and study it as an ecologist. "That hasn't happened as often as it should," he said.

Provided by Washington University in St. Louis