# Land use may weaken amphibian's capacity to fight infection and disease

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Scientists found changes in the microbiome and skin secretions of the cricket frog are linked to man-made changes to the environment and may make the animals more vulnerable to infection and disease. Credit: Tim Krynak

Man-made changes to the environment may be damaging the immune systems of a species of frog whose populations have drastically declined since the 1970s, according to a new study by researchers at Case Western Reserve University and the Holden Arboretum.

"These Blanchard's cricket frogs have nearly gone extinct in their northern range, so we're almost forensically trying to understand what happened," said Mike Benard, a biology professor at Case Western Reserve. "This study suggests that changes we are making to the environment have the potential to make animals more susceptible to diseases and therefore may lead to population declines."

Scientists found that habitat characteristics explained the differences in immune defense traits of frogs between populations. They found that the skin microbiomes ?symbiotic bacterial and fungal communities on the skin ?of frogs from disturbed sites, like residential and agricultural lands, were different from the skin microbiomes of frogs from more natural habitats. They also found natural peptide secretions—proteins frogs secrete from their skin that protect against pathogens—differed between frogs from different environments.

Both changes potentially alter the amphibian's immune defense capabilities.

These findings and more are published in the journal *Biological Conservation*.

Research is increasingly showing that microbiomes in the gut and on the skin and antimicrobial peptides excreted by humans and other animals play important roles in fighting infection and disease.

"We're seeing a lot of disease-related declines among amphibians, not to mention other groups of animals, such as bats plagued with white-nose

syndrome and bees suffering from colony collapse disorder," said Katherine Krynak, a postdoctoral scholar in Case Western Reserve's Department of Biology and leader of the study. "This research shows that land use—farming or treating lawns with herbicides, pesticides and fertilizers—can influence traits that protect animals from disease."

## Testing Blanchard cricket frogs

Blanchard's cricket frogs are about an inch long. They had once been widely spread across Wisconsin, Michigan and northern Ohio, but now only pockets remain in this northern region.

Frogs used in the study were from ponds in various habitats: natural ponds surrounded by forest or prairie, or more disturbed ponds surrounded by houses, on farmed land or near athletic fields, parking lots and golf courses. In addition to considering the physical differences, the researchers tested water chemistry and quality in each pond.

With permission from the states of Ohio and Michigan, Krynak, Benard and David Burke, a scientist and research chair at Holden Arboretum in Kirtland, Ohio, examined samples Krynak had collected from the frogs. Krynak used Q-tip like swabs to obtain samples of the skin microbiome, and then placed the frogs in a solution that gently induced the animals to secrete the antimicrobial peptides.

Krynak and Burke then used molecular methods to examine the community of microbes on the frogs' skin. Burke, who studies symbiotic interactions between plants and microbial communities, is also an adjunct assistant professor of biology at Case Western Reserve. Krynak and Burke also examined the amount of peptides the frogs produced and how effective the peptides were against an amphibian pathogen they cultured in the lab.

## What they found

The researchers found microbiome differences between frogs that live in natural areas, such as a pond owned by the Nature Conservancy, and those in ponds surrounded by highly "managed" land, such as farmland or residential properties.

"What we're seeing is the bacteria on the skin can vary markedly, depending on what people are doing to the environment that the frogs are living in," Burke said.

A pond's latitude, conductivity—a proxy for chemical runoff—and size also appear to affect the microbiome.

The amount of natural peptide secretions produced from the frogs' skin also varied across sites and was influenced by both the size of the pond and the conductivity of the water.

## At what cost?

Some of the skin secretions have been shown to fight off fungal infections, Krynak said. But in petri dishes in the lab, the growth rate of chytrid fungus, which has been linked to devastating population declines in amphibians worldwide, climbed with increasing Blanchard's cricket frog natural peptide secretions.

The researchers will further investigate why higher concentrations of peptides appear to allow the killer fungus to grow faster in this species.

"This pattern suggests that in areas where land use increases the amount of the peptides these frogs produce, this particular pathogen could have devastating effects" Krynak said.

The team will also look more directly at how the environment interacts with a population's genes, changing the expression of traits. "Not only may the environment be altering traits now, but it may be dampening the ability of a population to adapt in the future," Krynak said.

They are also experimentally isolating factors such as how a commonly used and commercially available glyphosate-based herbicide may alter these immune defense traits.

Environmental alteration of defense traits may explain why different amphibian populations show different levels of resistance to infection and disease.

Krynak said there's a strong chance that the environment is affecting these traits in other amphibians and wildlife in general.

"By improving our understanding of the factors influencing immune defense traits capabilities, we are given the opportunity to make changes to our land management practices to better protect wildlife health" she said "and in all likelihood, our own health as a consequence".

**More information:** *Biological Conservation*, dx.doi.org/10.1016/j.biocon.2015.11.019

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