

Studies offer new insights into causes of deformed frogs



Photo by Jay Bowerman
Rana cascadae that have lost hind limbs as a result of dragonfly predation.

By William Souder | Monday, Aug. 17, 2009

Remember Minnesota's famous deformed frogs? New studies from two groups of researchers working half a world apart have just added important insights into this tantalizing environmental puzzle — while leaving a full explanation still out of reach.

It's been 14 years since a group of middle-schoolers on a field trip discovered frogs with bizarre leg malformations at a farm pond near Henderson, in the Minnesota River Valley an hour southwest of the Twin Cities. Some of the amphibians had extra hind legs that appeared as pale, shrunken imitations of normal limbs. Some were missing legs, or had legs that were partial or misshapen. In the months that followed, frogs with the same and even worse limb deformities were discovered in dozens of wetlands across Minnesota and Wisconsin, and it was learned that similar outbreaks were already under investigation in Canada's Quebec province.

Of immediate concern was the possibility that the deformities were being caused by a water contaminant that could affect other species, including humans. By the spring of 1997, several federal agencies and a number of academic researchers were working on the problem.



Researchers eventually discovered that a species of tiny aquatic parasite called a trematode imbeds itself in the developing leg tissue of tadpoles and causes them to grow additional legs. Because much of the attention had focused on frogs with extra legs, the finding was widely reported as the end of the story.

Most reports involved missing or partial legs

But it wasn't. There were outbreaks of frogs with extra legs in which parasites were not the cause. More important, most of the hundreds of reports of deformed frogs involved missing or partial legs. Parasites were implicated in missing legs, too, but not at the high frequencies such animals were being discovered in the field.

Now research on frogs with missing legs in England and Oregon — undertaken independently but arriving at identical conclusions — has found a common cause of missing legs in frogs.

They're being eaten off.

The two studies are notable for how closely their findings parallel each other. In England, Brandon Ballengée of the University of Plymouth, studying common toads from ponds in Yorkshire, found that missing, partial and abnormal legs are caused by "selective predation" by dragonfly nymphs. Like frogs and toads, dragonflies lay eggs that hatch into aquatic larvae. Dragonfly nymphs, of which there are many, widely distributed species, are aggressive predators that can be an inch or more in length and are equipped with sharp mouth parts and a specialized grabbing appendage — think of the monster in "Alien" — that catches and holds prey.

Hind limbs most often affected

Although dragonfly nymphs are too small to eat entire tadpoles, when Ballengée studied them attacking tadpoles in the wild, and also under experimental conditions in aquaria, he found that they latched onto tadpoles and then searched for body parts they could consume before releasing their injured prey. The nymphs sometimes went after eyes or tails, but most often focused their attention on the hind limbs, which develop externally from small buds of tissue on either side of the tail. The resulting deformities mirrored the abnormalities seen in the field.

To amplify the study's findings, Ballengée's collaborator, Stanley Sessions, of Hartwick College in New York, examined the effects of leg amputation in developing frogs. Sessions, one of the original U.S. investigators of amphibian deformities, had been the first scientist to demonstrate a connection between parasites and extra legs — and had also suggested that predation might explain missing legs. Along with other experts in limb development, Sessions had suspected that the most mysterious abnormalities — leg stumps that were themselves abnormal — might result from incomplete attempts to regenerate a limb that had been chewed off.

Among the vertebrates — including humans and animals with an internal skeleton — only salamanders can regenerate a missing limb at any time in their lives. Frogs possess the power of regeneration as tadpoles, but their capacity to grow a new leg to replace a lost one diminishes as they approach metamorphosis, by which time it has disappeared altogether.

In his lab, Sessions amputated the hind limb at the knee from wood frog tadpoles at different developmental stages. He found that limbs lost early in development were completely re-grown, while later-stage amputations

produced a range of partial and deformed limbs that closely resemble those seen in deformed frogs in the wild. Ballengée and Sessions published their results this summer in the *Journal of Experimental Zoology*.

Field observations in Oregon

Meanwhile, very much the same thing was seen in several wetlands near Bend, in central Oregon. In a paper to be published later this year in the journal *Ecology*, Jay Bowerman, a researcher at the Sunriver Nature Center, and Pieter Johnson, a biologist at the University of Colorado, describe what they term "sub-lethal predation" on developing tadpoles by dragonfly nymphs and also stickleback fish. Sticklebacks are a thumb-sized fish with mouths that are tiny but crammed with needle-like teeth.

The Oregon field observations are important because they span a long time — Bowerman began observing toads with missing limbs in 1988 — and because the incidence of deformities on one frog species parallels the abundance and scarcity of sticklebacks. In years following heavy winter fish kills there are few sticklebacks and few missing legs in frogs. And vice versa. Toads and frogs at the Oregon sites also exhibited high frequencies of deformities — something that wasn't true in the English ponds.

Considered together, both studies offer answers to several key questions about the deformities seen in frogs for more than a decade. It is rare, for example, to find a missing front leg on a frog. Unlike the hind limbs, which are exposed as they begin to grow, the front legs develop within the chest cavity and only emerge at end of the tadpole phase, at metamorphosis. So if legs are being lost to predators, it makes sense that it's the hind limbs.

And while Sessions only amputated legs at the knee, he believes that legs bitten off at other points along their length, and at various times in development, can produce regeneration responses that can give rise to essentially all of the missing and partial limb types seen in the wild — all without leaving behind obvious signs of wounding or scarring.

Predation would also help to explain a feature of common leg deformities that's harder to reconcile with exposure to a chemical: Most frogs with missing hind legs are missing only one. A "unilateral" abnormality is easier to understand as the result of a predator attack.

New findings raise question about missing legs

At the same time, the new findings raise a question of their own. Since small aquatic predators routinely occur in the same wetlands as frogs, why aren't frogs with missing legs turning up everywhere? Ballengée says they probably do occur more commonly than anyone has yet documented. He said he's finding plenty of them on research trips in Canada this summer, and also among historical specimens in museum collections that go back decades. Still, there are uncounted wetlands across the landscape that harbor breeding populations of frogs, and only a handful of scientists who systematically monitor a minuscule fraction of them. If parasites and predators are causing widespread leg deformities in frogs, we know that by inference and not by direct observation.

For Bowerman, a low-key naturalist who may have been the first U.S. scientist to routinely track deformed amphibians, and for Johnson, who as an undergraduate did groundbreaking experiments to confirm the role of parasites in extra limbs, the predation findings add weight to the argument that deformed frogs are a regular feature of natural wetland

ecology. Sessions and Ballengée agree.

"When I got into this, I initially thought some kind of pollutant was involved," says Ballengée. He changed his mind, he says, when he observed in the field dragonfly nymphs "grazing" hungrily on toad tadpoles as they massed in the shallows just before metamorphosis. "I think we've found another big piece of the puzzle," says Ballengée. "Selective predation is the second of the two prime causes of deformities."

But the findings are sure to rankle critics, who don't question the role of either parasites or predators in some amphibian deformities but who have complained that both causes have been oversold as universal explanations for all outbreaks of abnormal limbs. When the Ballengée and Sessions paper was published, an online report by the BBC declared the legless frog mystery "solved," echoing the chorus of reports a decade ago declaring that all deformities were caused by parasites. Such expansive statements have caused consternation among scientists who worry about a precipitous worldwide decline in frog populations and what it may signal about a changing environment.

Extrapolations seen as unwise

Mike Lannoo, of the University of Indiana Terre Haute, is one of the country's leading herpetologists and author of the book "Malformed Frogs," a review of the deformed frog problem that concluded there are likely many causes of abnormal limbs in frogs. Lannoo laments media reports suggesting that parasites and predators are now the answer to all deformities. But he says he's not sure whether to blame the scientists reporting their results or the media covering those findings.

"I'm comfortable with these results in the context in which the work was done," says Lannoo. "I'm less comfortable extrapolating that to the level of global significance. Any global pronouncement requires a global data set. Just because a missing leg can be caused by a predator doesn't mean that every missing leg was caused by a predator."



Photo by Jay Bowerman
Portion of one frog, showing complete amputation, wound completely healed, following predation in the wild by dragonfly larva.

Proposing parasites and predators as a general explanation for frog deformities is a kind of "biology gone wild scenario" says Lannoo, one that masks other possible causes and potentially serious environmental degradation. There are, Lannoo argues, thousands of frog species in the world, and broad conclusions based on what happens to one or two species in a handful of wetlands are "tenuous at best." Lannoo says it's

important to remember that a host of chemicals — pesticides, hormone mimics, metals, PCBs — are known to cause developmental abnormalities in frogs, and lab studies of chemical exposure have produced frogs with deformities comparable to those in nature.

Pieter Johnson says he wishes scientists working on frog deformities would learn to get along. "My feeling has always been that it should be possible to work together on these questions," he says. "In a given situation it's pretty easy to rule out parasites. And it's pretty easy to rule out predators. Well, we've come across sites where neither is a factor. And those sites concern me."

Intersection of nature and human influences

Jay Bowerman thinks the role stickleback fish play in Oregon demonstrates how nature and human influences can intersect. Sticklebacks are not native to the portion of the Deschutes Rivers system where Sunriver is located. Since their introduction there several decades ago, probably by fishermen using them as bait, the stickleback population has exploded. "This is an invasive species whose numbers are all out of proportion," says Bowerman. "I don't think they normally like to feed on tadpoles. But they've adapted."

Stan Sessions says that testing water for chemicals is expensive and time consuming and frequently a dead end. So it makes sense, he says, to look first for the "cheap and easy" explanation of either parasites or predators. Lannoo agrees that screening for parasites at a deformities hot spot makes sense because parasites are "either present or not present" and therefore easily ruled out. Predators are different, Lannoo says, in that it is almost impossible to find a wetland with tadpoles that does not also have aquatic predators.

Sessions concedes that frogs with missing legs by themselves give up little information about what caused their abnormalities. "You just never know absolutely," Sessions says. "If someone hands you a frog with a missing leg you just cannot say for sure what caused it. But now you can make a good guess."

For Sessions, the mounting evidence for the twin mechanisms of parasites and predators as causes of deformities — an explanation he has long championed — is a vindication of sorts, though he will admit only the facts have led to where the facts have led. "I really think this is just an affirmation that science tends to work things out."

William Souder is a Minnesota journalist and the author of "A Plague of Frogs." His most recent book, "Under a Wild Sky," was a finalist for the Pulitzer Prize.

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