ScienceWatch-The Decline of Frogs

In the past 20 years reports around the world have documented the decline or outright extinction of many frog species, even in apparently pristine environments. More recently schoolchildren in Minnesota discovered frog populations with extra legs, missing limbs and other deformities. These reports have fueled speculation by environmentalists that frogs are like canaries in the coal mine. Canaries are extremely sensitive to deadly mine gasses and would be brought into the mine as early warning alarms. Frogs, the thinking goes, are especially vulnerable to ultraviolet light (UV) and environmental pollutants because they readily absorb substances through their skin, are vegetarians as tadpoles, become carnivores as adults, and live both on land and in water. Furthermore, as indicators of global health their decline may be a sign of a worldwide rise in UV radiation due to ozone depletion and increased pollutants caused by human activity. However, ten years of research has failed to show a conclusive link between environmental factors and major declines of frog populations.

Instead, evidence has recently mounted to indicate that frogs are suffering from a worldwide epidemic caused by emerging pathogens, i.e., pathogens that have learned new tricks to overcome host defenses. Emerging pathogens can wreak havoc until the host "learns" to defend itself (consider the AIDS epidemic). In the case of frogs the pathogens are a fungus and a parasitic flatworm (trematode). The microscopic, aquatic fungus, known as a chytrid, can be isolated from dead frogs in the field. Once healthy frogs are inoculated, they quickly die and the fungus can be recovered from their corpses. That experiment is the classic way to prove an organism causes a disease. Exactly how the fungus kills is unknown, perhaps it secretes a toxin, but its water-borne spores invade the skin where it apparently lives off the keratin and reproduces itself. The fungus has also been found in specimens preserved from earlier major die-offs in the United States, implicating it, rather than a pollutant, as the causative agent.

In Australia, other researchers documented extinctions of entire frog species in unpolluted streams where UV radiation had not increased. Common to all declining populations was a chytrid infection that first appears in the tadpole's mouth and, after metamorphosis, spreads throughout the frog's skin, killing it. The chytrid fungus (*Batrachochytrium dendrobatidis*), a new genus and species, has now spread across the entire Australian continent, killing frogs in its wake. It has also turned up on ten different frog species in Panama, and preliminary DNA fingerprinting results show the Panamanian pathogen to be the same species as the one devastating Australian populations. As if having to deal with a fungal pandemic weren't enough, frogs are also being attacked by a parasitic trematode that appears to be responsible for the abnormal limb development.

In the April 30th issue of *Science* researchers report a clear association between the trematode (*Ribeiroia sp.*) and severely abnormal Pacific treefrogs in California. A survey found that extra, partial or missing hindlimbs were common in four of the 13 ponds with treefrogs. Water testing failed to detect any pesticides, PCBs or heavy metals, but an aquatic snail was found in the ponds with abnormal frogs. These snails often serve as an

intermediate host for parasitic trematodes and the team speculated that trematode larvae (cercariae) released from the snails were subsequently infecting the frogs and causing the abnormalities. To prove this they collected eggs, hatched them and infected tadpoles with various doses of cercariae.

The results were unequivocal. As the parasitic load increased so did the rate of abnormal limb development. In batches with the highest load of 80 larvae per tadpole, all the frogs exhibited abnormalities and less than half survived. This contrasted with the control, lacking cercariae, where 88% survived and all were normal. How can the trematode induce such severe abnormalities? Earlier work by another researcher had shown that in salamanders, extra legs could result from implanting tiny beads in the developing limbs. Evidently, the beads could move the embryonic cells around, re-positioning them in the wrong areas. The microscopic cercariae infest the tadpoles around the limb buds and apparently produce the same mechanical displacement as the beads.

A second report in the same issue of *Science* directly addresses the role pesticides may play in the frog decline by examining the effect of retinoic acid on inducing abnormal limbs. This substance is part of a class of compounds known as retinoids, chemicals that are normally present in embryos, where they trigger limb development, and whose effects are mimicked by at least one pesticide. Could pesticides be the culprit? Apparently, they are not. The report shows that the kinds of abnormalities found in field-caught frogs are different from those induced by retinoic acid, but are the same as those caused by the bead implantation and trematode infestation described above. The researchers conclude that pesticides are not responsible for the increase in abnormal limb development. Instead, they say that frogs are suffering from a one-two punch delivered by a deforming parasite and a killer fungus.

While not necessarily helpful to the frogs, these studies show that by considering frogs to be the canaries of an increasingly fouled planet, environmentalists may have overreacted to reports of frog die-offs. However, many scientists still caution that environmental degradation may contribute to the appearance and spread of these pathogens and cannot be ignored. They say that healthy habitats should tip the balance in favor of the frogs and against the pathogens. So although it appears that croaking frogs can't match singing canaries, environmental degradation may still play a role in some die-offs.

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