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Amphibians as environmental sentinels

World-wide, there are believed to be 4,780 species of amphibians. Many of them have shown increasing signs of stress in recent years. Some species have disappeared, others are no longer found where they used to be and yet others have begun to exhibit an alarming incidence of deformities. All these events are telling us that something is going wrong. Until recently, much of the evidence on global amphibian decline has been anecdotal. Herpetologists were able to analyse data only from restricted regions and by extrapolating those data, were able to infer that a decline in amphibians was under way. Houlahan *et al* (2000) for the first time demonstrated conclusively a global fall in the population of amphibians, especially of frogs and salamanders, over the past 40 years: "The most serious population decline may have occurred long before scientists noticed and sounded the alarm". Their analysis concluded that the world's amphibian population has decreased by more than 50% since the 1950s. The decline was steepest in the 1960s – as much as 15% a year – but continued at a slower rate, roughly 2% a year in the 1980s and '90s; North American populations showed the most precipitous fall.

The global decline in amphibian populations, and more recently the disturbing number of deformed amphibians, has caused many researchers to believe that they may be early indicators of serious environmental problems. Species have been reported to have disappeared from Europe, America, Australia, Asia, Africa and elsewhere (Alford *et al* 2001). Various factors have been implicated in global amphibian declines. The animals are sensitive to pollution, because they live at the interface of two environments – land and water – and can easily absorb pollutants through the skin. The skin absorbs chemical contaminants from agricultural run-off that results from an indiscriminate use of pesticides, herbicides and fertilizers. Ionizing radiation (UV-B) resulting from ozone layer depletion, acid precipitation and the introduction of exotic competitors and predators are other hazardous factors. A new species of chytrid fungus has also been implicated in global amphibian decline (Halliday 1998; Rollins-Smith *et al* 2002). The fungus may exude a lethal toxin or suffocate frogs by clogging skin pores (Kaiser 1998). The deleterious effect of pollutants and chemicals have mostly been associated with finding frogs with missing legs, extra legs, misshapen legs, paralyzed legs that stuck out from the body at odd places, legs that were webbed together with extra skin, legs that were fused to the body, and legs that split into two half-way; frogs have also been found with missing eyes and with one external eye, the second eye growing inside the throat (Cohen 2001). Researchers have discovered that the level of nitrogen-based compounds which the United States Environment Protection Agency (EPA) found in agricultural areas as a result of using crop fertilizers is enough to kill some species of amphibians, especially at their more vulnerable larval stages (Blaustein 2001). When exposed to moderate amounts of nitrates and nitrites, some tadpoles and young frogs reduce their feeding activity, swim less vigorously, experience disequilibrium, develop physical abnormalities, suffer paralysis and eventually die.

Now a report on one of the top-selling weed killers, atrazine, shows that it affects sexual development in frogs at concentrations 30 times lower than that allowed by the EPA, thereby raising concerns about the heavy use of this herbicide (Hayes *et al* 2002). Hayes and co-workers have showed that atrazine demasculinizes tadpoles and turns them into hermaphrodites. The herbicide also lowers levels of the male hormone testosterone in sexually mature male frogs by a factor of 10, to levels lower than those in normal female frogs. It is unclear whether these abnormalities lead to reduced fertility.

Hayes *et al* (2002) used the African clawed frog, *Xenopus laevis*, which is very sensitive to hormones that mimic the effect of endogenous sex hormones. Using two separate populations of frogs raised in three separate tanks, experiments (replicated 51 times) were set up with various concentrations of atrazine. They found atrazine to affect the sexual development of frogs at concentrations of 0·1 ppb (parts per billion) and higher, which is 30 times lower than the allowable limit of 3 ppb in drinking water and 120 times lower than the proposed chronic exposure limit for aquatic life which is 12 ppb. At these concentrations, as many as 16% of the animals had more than the normal numbers of gonads – including one animal with six testes – or had both male and female organs (testes and ovaries). No control animal had such abnormalities. The development of vocal sacs showed similar results. Normal males at metamorphosis have larger vocal organs than females. The organs of more than 80% of males exposed to 1 ppb or more of atrazine were smaller than average. Sexually mature males showed a 10-fold decrease in testosterone levels, bringing them below levels found in normal females. This suggests that atrazine acts by disrupting the synthesis of sex hormones, a hypothesis which could also explain the smaller than normal larynges and abnormal gonads. Hayes and his colleagues conducted a survey of atrazine-contaminated ponds in Midwestern agricultural lands in the USA to see if such reproductive abnormalities occur in frogs in the wild. They turned up many native leopard frogs (*Rana pipiens*) with similar problems, and are now testing captured animals to determine whether these changes are due to atrazine. Levels of 40 ppb atrazine have been measured in rain and spring water in parts of the US Midwest, while atrazine in agricultural runoff can be present at several parts per million. The authors doubt that atrazine has such severe effects on humans, because the herbicide does not accumulate in our tissues and humans, unlike frogs, do not spend their lives in water. Nevertheless, the effects of atrazine on frogs could be a sign that the herbicide is subtly affecting human sex hormones, too, interfering with androgens, such as testosterone, that control male sexual characteristics.

The data raise new concerns for amphibians with regard to atrazine. The findings come at a time when allowable levels of atrazine in drinking water, which stand today at 3 ppb, are being re-evaluated. While much remains to be understood about the causes behind what is going on, e.g. whether atrazine is the sole, or even major, culprit and what the implications are for food webs in general and humans in particular, the report by Hayes *et al* (2002) has already re-ignited the long-standing debate on the exact cause of amphibian decline. The worrisome prospect is, of course, that we may be seeing an early warning of serious world-wide ecosystem imbalance. The health of amphibians is closely linked to the health of the environment, and these animals are early indicators of significant environmental changes that may otherwise go undetected by humans. They are fortuitous canaries in our coal mine (Halliday 2000). The burden of proof has now shifted to those who would continue to ignore their fate.

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