STEPHEN AUSMUS (K10009-1)

North Dakota State University students Paula Fernholz and Mark Davis determine the weight of a Northern Leopard tadpole *(Rana pipiens)* undergoing metamorphosis.

It's Not Easy Being Green

Seeking the Cause of Frog Malformations

STEPHEN AUSMUS (K10012-1)

A Northern Leopard frog with an abnormal spine (see hump) and a missing right eye (anophthalmia), two of the significant malformations observed in frog populations.

Living in a pond may not seem ideal for you or me, but frogs love it. But sometimes life in a pond can serve them up some nasty surprises.

During a field trip in 1995, kids from a Minnesota middle school discovered unusual characteristics in some frogs. In fact, quite a few frogs showed significant malformations: missing limbs, extra limbs, and missing eyes were all observed by the group.

Initially, agricultural chemicals were thought to be the possible cause of such malformations. But other factors soon moved to the front of the list.

In recent years, biologists have noticed a decline in frog populations and an increase in deformed frogs. Could these frogs be sentinels, just as canaries once warned coal miners of unsafe air conditions? Are the frogs telling us that some of the water we drink or the food we eat in our increasingly urbanized world is contaminated with certain environmental pollutants that may harm public health? Whether or not this is so or what constitutes an environmental hazard are subjects of research by a group of five ARS scientists, headed by Gerald L. Larsen, at the Red River Valley Agricultural Research Center, Fargo, North Dakota.

A Frog's Life

Frogs are amphibious creatures, whose skin acts not only as a covering, but also as part of its respiratory system. The semi-permeability of the skin makes gas transport efficient but allows other substances to enter the body as well. Also, from fertilized eggs through tadpole metamorphosis, frogs are virtually in continuous contact with the water.

Former ARS biologist Eric A. E. Garber scrutinized water samples from Minnesota ponds to find out whether mineral deposits and hormonally active agents, such as naturally occurring phytoestrogens or estradiol, may be involved in frog malformations. "The concern is that some substances in water may have long-term and more complicated effects and could enter the food chain via water," Garber says.

As hypotheses abound in the scientific community concerning the affected

frogs, the Fargo team collaborates with other researchers, including the Minnesota Pollution Control Agency (MPCA), to find out whether deformed northern leopard frogs at certain sites are victims of endocrine-disrupting compounds. Synthetic and naturally occurring compounds from known and unknown sources may find their way into the environment through sewage or animal manure, and their concentrations may be affected by the movement of water through soils.

Joseph Magner, MPCA's senior hydrologist, is certain there are multiple factors at work. "No silver bullet has been found and no one particular factor explains all the malformations in the frogs."

Tools of the Trade

Larsen and his team used the latest technologies to analyze water samples from Minnesota lakes for the presence of endocrine-disrupting compounds, such as estradiol, diethylstilbestrol, and the isoflavone genistein.

At several sites, researchers found relationships among estrogenic (estradiol-like) activity, mineral levels in the water, and the occurrence of malformed frogs. Judith L. Erb, of ThreeFold Sensors, Inc., Ann Arbor, Michigan, tested water samples using a fiber optic biosensor and found a correlation between the presence of estrogen and malformations.

"All water samples from sites that contained malformed frogs exhibited elevated estrogenic activity," says Erb. "But while the technology can detect estrogenic activity, it can't identify which estrogens are present." In further research on estrogenic activity, the Fargo team confirmed the presence of estrogens and has developed the technology to isolate, identify, and purify the chemicals that prove to be important.

Two compounds, estradiol and testosterone, are potent endocrine disruptors that are present in large quantities in animal waste and are readily mobile in the environment. "Our findings from soil and compost studies indicate that estradiol and testosterone are not completely degraded in the environment and that they have the potential to enter ground and surface waters," says Heldur Hakk, a chemist with the Fargo team. Another team member, physiologist Nancy Shappell, is examining the efficiency of water treatment plants in removing estrogens.

While estrogens seem to be involved, Larsen's group also noticed that many sites were deficient in key minerals such as sodium and potassium. The concentrations were not low enough to kill tadpoles, but they were low enough to delay development in laboratory experiments. Such a delay could shift and lengthen windows of susceptibility in which malformation-triggering events, such as flatworm infections or endocrine disruption, may occur.

The mineral level fluctuations can be explained by examining meteorological records. In the late 1980s and early 1990s, there was a record drought, followed by a record wet period. From 1995 to 1997—the end of the wet period—incidences of frog malformations peaked in Minnesota. Researchers speculated that the wet period might have opened new pathways of water movement, which mobilized chemical and biological agents and decreased the concentration of minerals in wetlands. Such meteorological events could help explain the sudden increase in malformed frogs over such a large and diverse region.

To analyze the water samples and study suspected compounds, scientists use a test known as the frog embryo teratogenesis assay: *Xenopus,* or FETAX for short. This test gauges mortality, malformation rate, and growth inhibition during the first 96 hours of development. During early stages of tadpole development, several oxidases—enzymes that detoxify compounds—are absent. Because tadpoles are vertebrate embryos that develop in water, FETAX provides a unique opportunity to study possible effects of environmental toxins on the developing embryos.

While the search continues, it seems that multiple factors play a role in frog deformities. "Recent research has linked the infection of a flatworm parasite called *Ribeiroia ondatrae* to amphibian malformations," says Larsen. "We are looking more closely at how this might occur."—By **Sharon Durham,** ARS.

This research is part of Food Safety, an ARS National Program (#108) described on the World Wide Web at http://www.nps.ars.usda.gov.

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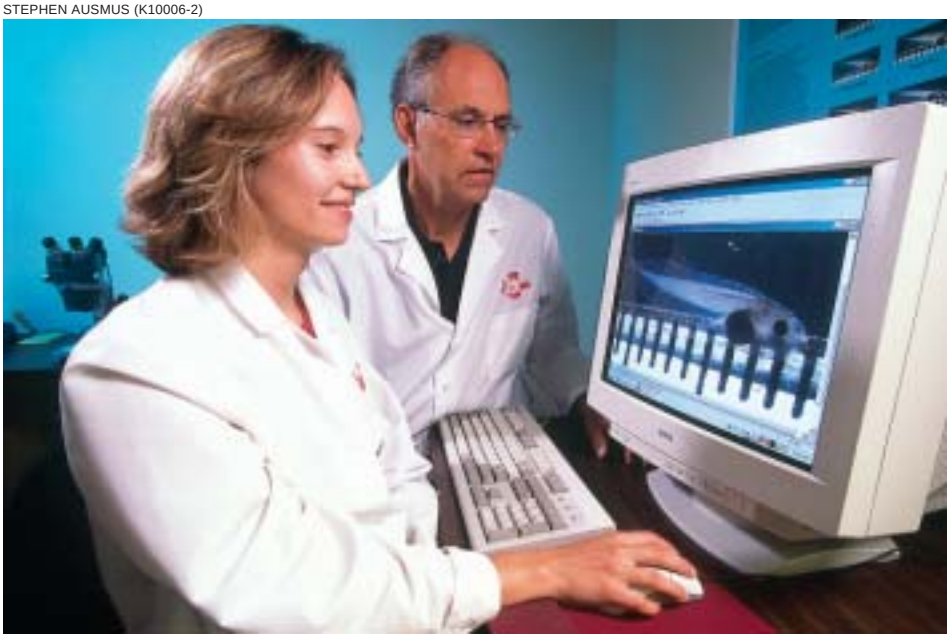

Technician Amy McGarvey and research leader Gerald Larsen measure tadpoles in the final step of the FETAX bioassay using a computer program and digital images.