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Malformed frogs in Minnesota: an update


Photo by David Hoppe

Background

Malformed frogs first became the topic of national news in August 1995 when students at a middle school in southern Minnesota discovered one-half of all the frogs they caught in a nearby pond were malformed. Since then, malformed frogs have been reported throughout Minnesota and elsewhere in the United States and Canada (http://www.npwrc.usgs.gov/narcam), and in other countries. The Minnesota Pollution Control Agency (MPCA) has been leading much of the research to discover the cause(s) for these malformations in Minnesota. The U.S. Geological Survey has been collaborating in this effort with studies on virus, bacteria, and parasite identification; water and sediment chemistry; hydrology and land use. Scientists from numerous federal agencies and universities also have been working with MPCA to find causes for this problem (see list on page 3). A chronicle of this effort conducted within Minnesota and elsewhere is presented in a book titled “A Plague of Frogs: the Horrifying True Story” (Souder, 2000) and in the Minnesota Conservation Volunteer (Souder, 2001). While malformed frogs also have been found in many other states, numerous studies have focused on malformed frogs in Minnesota, and significant progress toward finding a cause or causes for malformation has been made. This Fact Sheet summarizes scientific results from malformed-frog studies conducted primarily in Minnesota.

Field Survey of Minnesota Frogs

From 1995 through 2000, nearly 25,000 frogs from 195 sites in Minnesota have been caught, examined, and released by MPCA scientists and collaborators, according to the MPCA Malformed Frogs database (Murzyn, 2000). Malformed frogs have been found throughout Minnesota (fig. 1). Malformations were found in 8 species of frogs and toads. For northern leopard frogs (*Rana pipiens*), the species most commonly found in Minnesota, 6.5 percent of 13,763 frogs collected were malformed. Malformations included missing limbs, missing digits, extra limbs, partial limbs, skin webbing, malformed jaws, and missing or extra eyes (Helgen and others, 1998; Canfield and others, 2000; Meteyer, Loeffler, and others, 2000). At some sites, many malformed frogs had “bony triangles” (bone bridges) in X-ray images, an abnormal development that also has been induced in frogs exposed to retinoid chemicals in the laboratory (Gardiner and Hoppe, 1999). The ends of partially developed bones often had a spongy appearance in X-ray images (Lannoo and others, 2001); this differs from what is seen when a predator causes partial limbs, indicating that attacks by predators did not cause those missing or partial limbs (Lannoo, 2000). Nearly all malformed frogs found since 1995 have been juveniles (Hoppe, 2001; Lannoo, 2000), indicating that malformed frogs rarely survive to become adults. This lack of survival may be contributing to the population decline reported for some amphibian species.

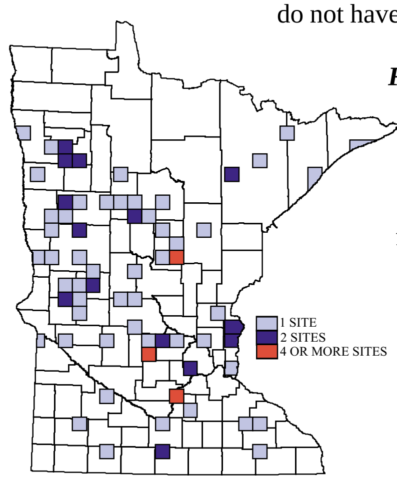

Figure 1: Locations where malformed frogs have been found in Minnesota, 1995-2000 (from Murzyn, 2000)

Historical malformation rates for frogs

In the past, malformed frogs were rarely found in Minnesota. Only 0.2 percent of 2,433 frog specimens collected in Minnesota during 1958-63 that are archived at the Bell Museum of Natural History at the University of Minnesota were malformed (Hoppe, 2000). Another 0.5 percent were abnormal due to predator attacks. Hoppe (2000) reported that only 0.2 percent of 1,772 frogs from nine field sites in west-central Minnesota during 1973-93 were malformed or were missing limbs from predator attack. In contrast, during 1996-97 Hoppe (2000) found that 2.3 percent of 2,548 frogs from those same nine sites were malformed (site-specific malformation percentages ranged from 0 to 9.4). Burkhart and others (2000) indicated that it is difficult to find sites that do not have malformed frogs.

Frog malformations at abnormal sites

At some sites in Minnesota the percentages of malformed frogs have been much higher since 1995. Twenty of the 195 MPCA sites have frequencies of frog malformations in excess of 5 percent and are termed “abnormal” sites. Some abnormal sites have malformation rates far in excess of 5 percent. At one site, for example, 25 percent of juvenile northern leopard frogs were malformed. At another site, 68 percent of all juvenile mink frogs (*Rana septentrionalis*) were malformed (Hoppe, 2001). The frequency of malformations peaked at different times of the year at different sites, generally from early August to early October (fig. 2)

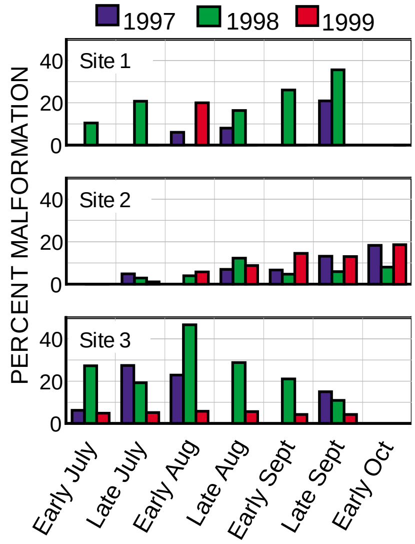

Figure 2: Malformation rates peak at different times from year to year and at different times from site to site (Murzyn, 2000).

(Canfield and others, 2000). The types of malformations at these abnormal sites differed from site to site as well (Meteyer, Loeffler, and others, 2000), suggesting multiple causes or combinations of causes for malformations. Missing limbs were the predominant malformation at one abnormal site, skin webbings were the most common malformation at another, and malformations of several types were observed at yet another abnormal site (fig. 3). Many of the malformation types recorded since 1995 have not been observed previously by Minnesota herpetologists (Hoppe, 2000). At a few of the abnormal sites, the percentage of malformed frogs has declined in recent years, but so has the total number of frogs (Canfield and others, 2000). Severe population die-offs at some of these sites have made it difficult to conduct studies on causes for malformation because it has become difficult to find frogs, especially northern leopard frogs (Shappell and others, 2000; Hoppe, 2001). A virus was found to be responsible for some of the die-offs (Meteyer, Cole, and others, 2000).

Abnormal sites exist in many different types of ponds and wetlands in Minnesota (fig. 4), but certain characteristics of some of the abnormal sites are noteworthy. Three of the abnormal sites were constructed wetlands, with little aquatic vegetation, firm sediments, and sometimes steeply sloping shorelines. Two of the worst abnormal sites have water that contains fewer dissolved minerals than most Minnesota wetlands (Jones and others, 2000). Some scientists speculated that ground-water contamination may have been contributing to the frog malformations at some of the abnormal sites. However, follow-up research on the hydrology of some of the abnormal sites indicates there is a notable lack of ground water flowing into those ponds (Jones and others, 1999).

Suspected causes for malformed frogs

Parasites

Many frog malformations in the western United States appear to be caused by the parasite *Ribeiroia ondatrae* (Johnson and others, 1999; Johnson and Lunde, 1999), a trematode (flatworm) that develops inside aquatic snails before burrowing into tadpoles (Johnson and others, 2001). Several species of amphibians developed high frequencies (40 to 100 percent) of severe limb malformations when exposed to *Ribeiroia* in the laboratory (Johnson and others, 2001). Other researchers have simulated the effects of parasite infection, demonstrating that malformation can result from simply inserting tiny beads into tadpoles at the location where hind limbs form (Sessions and Ruth, 1990). At several of the abnormal sites in Minnesota, the frogs are heavily infected with *Ribeiroia* (Sutherland, 2001); however, *Ribeiroia* has not been found at other abnormal sites in Minnesota (Sutherland, 2001), indicating that other factors are causing malformations at those abnormal sites. It is not clear why *Ribeiroia* infection may have increased in recent years, but there is some evidence that higher levels of nutrients may promote the parasite's life cycle (Johnson and others, 2001).

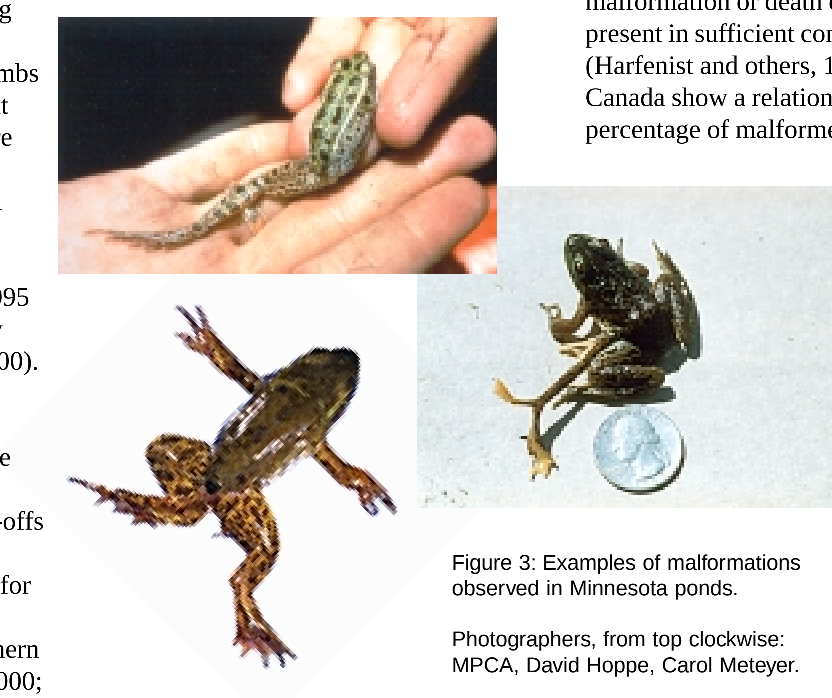

Figure 3: Examples of malformations observed in Minnesota ponds.

Photographers, from top clockwise: MPCA, David Hoppe, Carol Meteyer.

Chemicals

Human-made chemicals often are suspected as the cause for frog malformation in Minnesota. Chemical analysis of water samples from most of the abnormal sites in Minnesota indicated no unusual concentrations for dozens of compounds analyzed by the Minnesota Department of Health (Helgen and others, 1998) and the National Institute for Environmental Health Sciences (Burkhart and others, 1998).

Pesticides are known to cause malformation or death of frogs when present in sufficient concentrations (Harfenist and others, 1989). Studies in Canada show a relation between the percentage of malformed frogs and pesticide use (Ouellet and others, 1997; Bishop and others, 1999). At the abnormal sites in Minnesota, few pesticides or degradation products were detected in water and sediment samples, and those detected were present in very small quantities (Jones and others, 1999). Methoprene, an insecticide widely used to control mosquitoes, also has been suspected as having caused malformations. Laboratory studies on northern leopard frogs by the Environmental Protection Agency indicated no induced malformations at methoprene concentrations commonly found in the environment (Ankley and others, 1998). In contrast, field trials of methoprene applied at mosquito-control concentrations did result in malformations among southern leopard frogs, a species not found in Minnesota (Sparling, 2000).

Endocrine disruptors also are being studied to determine if they are responsible for some of the frog malformations in Minnesota. Endocrine disruptors are natural and human-made chemicals that interfere with or mimic natural hormones that control development, growth, and behavior of organisms. The number of endocrine disruptors is unknown; only during the last decade has screening of chemicals begun to evaluate endocrine-disrupting activity. Environmental retinoids are one class of endocrine disruptors that has received special attention in the frog malformation studies. Retinoic acid has been shown to induce frog malformations in the laboratory (Bryant and Gardiner, 1992). Retinoid activity has been identified in some of the ponds in Minnesota (Gardiner and Hoppe, 1999), but so far no proof exists that the concentrations detected can induce frog malformations in natural settings.

Estrogens are another class of endocrine disruptors under study. Although normally associated with sexual development, estrogens at high concentrations also can affect limb development (Hayes, 2000). Garber and others (2001) used two methods to detect environmental estrogens at many of the abnormal sites in Minnesota and found a relation between the concentrations of estrogenic compounds and the percentages of malformed frogs. They also found low concentrations of some minerals (sodium, potassium) in some of these ponds where estrogens were found and suggested that this combination of factors may be related to frog malformation at sites where parasites were not found in malformed frogs.

Ultraviolet light

The amount of ultraviolet (UV) light reaching the Earth's surface is increasing with time, and current amounts of UV radiation have been shown to cause malformations in frogs in the laboratory (Hays and others, 1996; Ankley and others, 1998). Light penetration in water is affected by shading from trees and aquatic plants, and by the amount and quality of dissolved organic carbon (Little, 1999). Research at abnormal sites in Minnesota indicates that the amount of UV radiation penetrating to the 10-centimeter depth in pond water is related to the percentage of malformed frogs in the pond (Little, 1999). Some scientists theorize that human-made or natural organic compounds present in Minnesota wetlands become toxic when activated by UV radiation (Burkhart and others, 1998; Little and Bridges, 2001).

Combinations of factors

It is likely that one or more combinations of chemicals, biological, and physical factors are responsible for causing the malformations in Minnesota frogs (Fort and others, 1999; Burkhart and others, 2000; Lannoo, 2000). Chemical combinations may be mixtures of natural and human-made organic chemicals, each of which is harmless on its own but toxic when combined (Burkhart and others, 2000). The number of possible combinations of chemicals, biological, and physical factors is enormous, which may explain why finding the causes for frog malformations has been a difficult task.

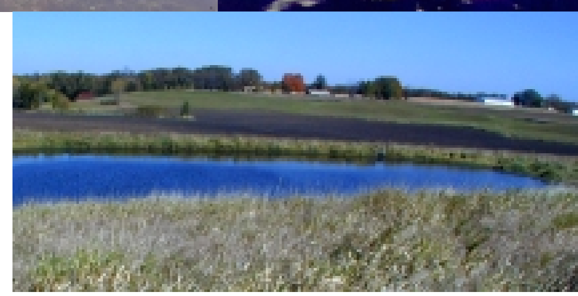

Figure 4: High rates of frog malformations have been found in a variety of ponds and wetlands.

Photographers from top clockwise: Eric Garber, Perry Jones, Eric Garber.

A continuing cooperative effort

Scientists have found malformed frogs throughout Minnesota. The incidence of malformation since 1995 exceeds historical malformation rates. Parasites cause some or most of the malformations at some, but not all of the sites. Many questions persist, and more work is needed to understand the causes for malformation that remain unexplained. Scientists from many different disciplines and organizations continue to work on the Minnesota malformed-frog mystery, both in laboratory studies and in the field. The MPCA is coordinating this research, and continues to collect much of the data on frog populations throughout the State. The following organizations have participated in this research:

Ball State University	Claremont McKenna College, Roberts Environmental Center	Hamline University
Indiana University, School of Medicine	Minnesota Pollution Control Agency	National Institute of Environmental Health Sciences
U.S. Department of Agriculture, Agricultural Research Service	U.S. Environmental Protection Agency	U.S. Fish and Wildlife Service
U.S. Geological Survey	University of California, Irvine	University of Illinois, Champaign-Urbana
University of Minnesota, Duluth, Minneapolis, St. Paul, Morris	University of Wisconsin, LaCrosse	Whittier College

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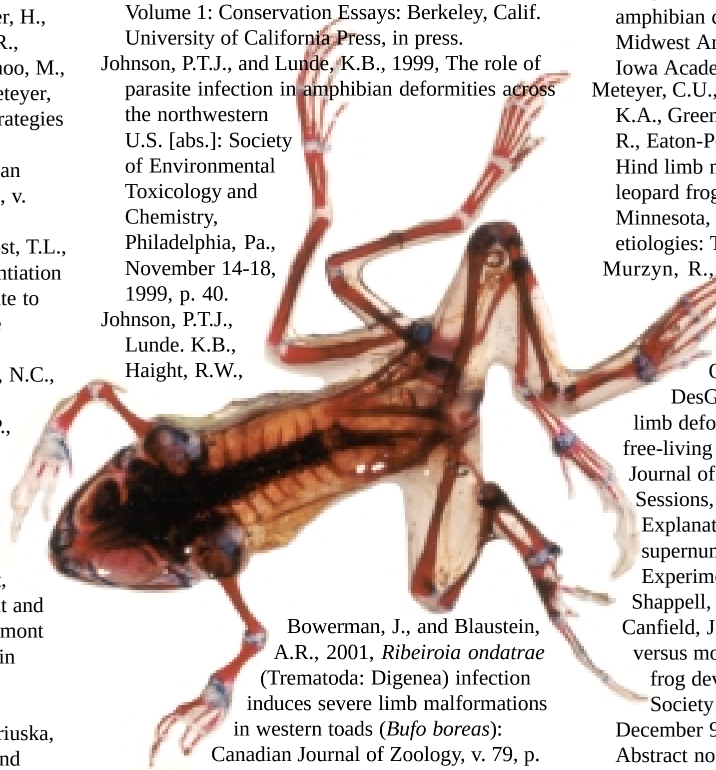

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