The Need for Water Quality Criteria for Frogs

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Amphibians are considered reliable indicators of environmental quality. In the western United States, a general decline of frog populations parallels an apparent worldwide decline. The factors thought to be contributing to declines in frog populations include habitat loss, introduction of exotic species, overexploitation, disease, climate change, and decreasing water quality. With respect to water quality, agroecosystems use 80–90% of the water resources in the western United States, frequently resulting in highly eutrophic conditions. Recent investigations suggest that these eutrophic conditions (elevated pH, water temperature, and un-ionized ammonia) may be associated with frog embryo mortality or malformations. However, water quality criteria for frogs and other amphibians do not currently exist. Here, we briefly review data that support the need to develop water quality parameters for frogs in agroecosystems and other habitats. *Key words*: agroecosystems, amphibian populations, frogs, pollution, water quality. *Environ Health Perspect* 103:352–357 (1995)

Water contamination and poor water quality in general have escalated in recent years (*1,2*). Concerns about alterations in water quality have increased as the need to share water among different consumers, including wildlife, has risen. Water quality needs of wildlife have often been neglected; this neglect is particularly true for amphibians (*3*).

There are several reasons for this neglect. Amphibians are not generally viewed as "cute and cuddly," and therefore, they enjoy limited popularity with the public (*4*). A lack of public regard and economic significance has been paralleled by a lack of research funding. Furthermore, past ecological studies have often focused solely on terrestrial or aquatic organisms, neglecting amphibians, which frequently occupy both terrestrial and aquatic habitats (*5*). Amphibians are considered reliable indicators of environmental quality (*6*). The early life stages (egg and larval) of many species are restricted to the aquatic environment, and many adults respire through a moist skin (*7*). Consequently, all life stages of amphibians are susceptible to dermal absorption of toxicants in water. Ingestion of contaminated prey is also a potential pathway for toxicants to enter amphibians (*7*). Amphibians can be major contributors to biomass and biodiversity within ecosystems; many adults are predators, embryos are prey for other trophic levels, and larvae are both herbivorous grazers and prey (*5,8,9*). Recent data suggest that a worldwide decline of amphibians is occurring (*3,10–13*).

Factors Contributing to the Decline of Frog Populations

Several reasons for the perceived decline in frog populations have been suggested (*6,8,10,11,13–16*). The most widely recognized and documented is habitat loss (*6,9,14,15*). For example, the contiguous United States are thought to have contained approximately 60–75 million ha of wetlands (*17*). During the 1950s to 1970s, 185,000 ha/year or 8.5% of these wetlands were drained. Currently, an estimated 43.7 million ha or between 58 and 73% of wetlands remain in the continental United States (*17*). Nearly 120,000 ha of wetlands are lost per year (*18,19*).

Within the remaining wetland habitats, numerous factors may be contributing to the decline of frog populations, such as the introduction of exotic species that may outcompete indigenous species for food and breeding sites or that may prey upon the indigenous species (*20,21*). For example, Hayes and Jennings (*21*) hypothesize that exotic fish species introduced into the waters of California may have foraging behaviors that increased predation upon the eggs and tadpoles of native ranid frog species. They note that catfish (*Ictaluridae spp.*) and sunfish (*Centrarchidae spp.*) usually forage by stirring the sediments and aquatic plants, locations in which ranid tadpoles are often found. And these researchers observed that in areas in which the catfish and sunfish have been introduced, native ranid populations have declined (*21*). Bradford (*22*) found declines of native frog populations in lakes of the Sierra Nevada Mountains of California when rainbow trout (*Salmo gairdneri*) and possibly golden trout (*Salmo aguabonita*), along with brook charr (*Salvelinus fontinalis*), were introduced. In other regions of the country, the largemouth bass (*Micropterus salmoides)* has been introduced for recreational fishing. This omnivorous fish ingests amphibian eggs and larvae, as well as adults (*23*).

Disease is another factor suspected of contributing to declines of frog populations. Opportunistic pathogens may overwhelm native species in a short time, or noninfectious disease can enter frogs via their permeable skin (*24–28*). During the 1970s and early 1980s, the North American leopard frog (*Rana pipiens*) (Fig. 1) suffered dramatic declines in many locations, not only in the United States, but in Canada and Mexico as well (*24,25*). Although the reasons for the mortality remain unknown, a current hypothesis is that disease may have been responsible. A condition known as "red leg" may have spread from population to population across the continent. Red leg is a syndrome characterized by kidney failure, ulceration, and hemorrhaged blood vessels. The latter are particularly prevalent on the ventral surface of the hind limbs and give the syndrome its name. Red leg usually results from an infection caused by the gram-negative pathogen, *Aeromonas hydrophilia*; however, it may be caused by other pathogens as well (*25*).

Declines in other amphibian populations, for example, the boreal toad (*Bufo boreas boreas*), which was extirpated from Colorado during the early 1980s, may also have resulted from this opportunistic pathogen (*27*). And in Oregon, a population of the western toad (*Bufo boreas*) has suffered high egg mortality since 1989. A mold, *Saprolegnia ferax*, which is commonly found in fresh water throughout the world, has been identified as the pathogen responsible for the egg mortality (*28*). Research on the numerous other viruses and bacteria that may infect amphibians and contribute to declining populations is lacking (*25*).

The effects of global climate change on amphibian populations is currently under investigation (*29*; Hansen L, personal communication). Recent research has demonstrated decreased hatching success of eggs of some amphibian species associated with increased levels of ultraviolet (UV) radiation, specifically UV-B radiation (290–320 nm light), whereas other species seem to be less affected (*29*). Blaustein et al. (*29*) placed eggs of three species of amphibians in cages in the water of a lake in the Cascade Mountains of Oregon and found that one species, the pacific tree frog (*Hyla regilla*), had high levels of photolyase activity, which protects the eggs from the UV-B, while the other two species, the cascade frog (*Rana cascadae*) (Fig. 2) and western toad, had little photolyase activity and suffered higher mortality than the tree frog. The different

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responses observed among species of amphibians may explain why some species have declined in population size while other species have not, even though all reside within the same region.

Other human-related activities may also be contributing to worldwide declines of amphibians. For example, in parts of India, overexploitation of frog populations for export as gourmet food has directly reduced populations (*30,31*). The concurrent loss of amphibian predators of insect pests coincides with increased pest populations, increased use of pesticides, and a subsequent deterioration in water quality (*30*). And finally, deteriorating water quality per se may be resulting in amphibian population declines.

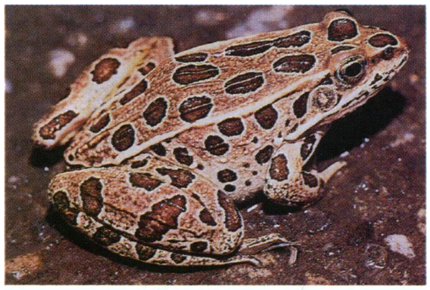

Figure 1. Adult northern leopard frog (*Rana pipiens*) of North America. During the 1970s, this species suffered an extensive decline that may have been caused by disease. [Photograph reproduced with permission from the Seattle Audubon Society (*99*).]

Figure 2. Adult cascade frog (*Rana cascadae*). Recent studies indicate susceptibility of this species to DNA damage in embryos from exposure to UV-B. [Photograph reproduced with permission from the Seattle Audubon Society (*99*).]

Figuure 3. Adult African clawed frog (*Xenopus laevis*). This nonindigenous species is often used in laboratory research (FETAX) and was used to evaluate water quality at the Klamath Basin National Wildlife Refuges.

Alteration of Water Quality

Sensitivity of frogs to aquatic toxicants compared to other species. Historically, aquatic toxicology has focused on concerns for fishes and invertebrates (*32*). However, some studies have compared the responses of amphibians to other aquatic species and found that amphibians are as sensitive, and often more sensitive, than other species when exposed to aquatic contaminants (*3,33–35*). For example, Holcombe et al. (*34*) exposed tadpoles of the African clawed frog (*Xenopus laevis*) (Fig. 3) and seven other aquatic species, among them the daphnid *Daphnia magna*, rainbow trout, fathead minnow (*Pimephales promelas*), and a midge (*Tanytarsus dissimilis*). The tadpole was the most sensitive species for one of the four compounds to which it was exposed, less sensitive than the trout, but more sensitive than the fathead minnow to one of the other three compounds (*34*).

Standardized methods to assess impact of aquatic contaminants on frogs in the laboratory have only recently been developed. Birge et al. (*36,37*) were among the first to use frog embryos as bioassay organisms. And in the early 1980s, Dumont et al. (*38*) developed the frog embryo teratogenesis assay-*Xenopus* (FETAX) (Fig. 4). This bioassay was originally developed as an alternative to mammalian testing of pharmacological compounds to assess teratogenesis (*39*). During the mid 1980s, Bantle and co-workers (*40,41*) used the FETAX bioassay to assess aquatic contamination. In 1991, the FETAX bioassay became the first amphibian bioassay accepted by American Society for Testing and Materials (*42*). Currently, this bioassay is in the process of becoming the first standardized amphibian bioassay approved for assessment of amphibian responses to water-borne contaminants by the U.S. Environmental Protection Agency (*43,44*).

Acidification. Previous studies of water quality in relation to amphibians have focused on acidification (*6,45–48*). Beattie and Tyler-Jones (*49*) found that low pH inhibited fertilization and embryonic development of the common frog *(Rana temporaria)*. They found that acidic environments can alter the physiological ionic balance in amphibians and reduce their growth and survival. Other researchers have studied low pH in relation to the mobilization of aluminum from sediments and observed decreased embryo survival in numerous species of amphibians (*50*; Rowe-Krumdick S, unpublished data). In the central United States, researchers used FETAX to assess heavy metal contamination from mine tailings that entered acidic waters; many embryos died, and those that did survive were severely malformed (*51*). Research on the effects of acidification of lakes and other waterways has been conducted primarily in the eastern United States, where increasing acidification of ponds associated with acid rainfall may be contributing to declining amphibian populations (*6,48,50*). Although the western part of the country has been less of a focus for these studies, concern over acidification in high-altitude lakes is growing (*52–55*).

Contaminants in agroecosystems in the western United States. Agricultural needs consume 65% of the available fresh water worldwide (*56*). In the western part of the United States, 80–90% of the water resources are delegated to agricultural uses (*57,58*). Agricultural lands in the West are intensely managed ecosystems; the soils are often tilled and modified with fertilizers and pesticides. Irrigation is widespread, occurring in specific areas according to contract schedules that are purchased by participating farmers (*57*). The water may be shared and recycled among growers and then channelled away from the fields for disposal in a river, stream, or reservoir.

The use of chemicals in agricultural production has escalated since the late-1940s (*59*). In 1960, synthetic organic pesticide production in the United States was 259 million kg (*60*). By 1986, 500 million kg of pesticides was applied to agricultural fields in the United States (*61*). Recently, 31 of the 50 states plus the Virgin Islands and Puerto Rico have reported concerns about groundwater contamination by pesticides to the EPA (*60*). Furthermore, the EPA has stated that agricultural runoff of pesticides and fertilizers contributes to the current nonpoint source pollution of fresh water in this country (*62*).

As noted previously, few studies on amphibian responses to pesticides have been conducted, and the results of these studies of single compounds have documented effects that range from temporary and reversible to delayed growth and death (*33,63*). Research on amphibians exposed to organochlorine compounds, which were widely applied to fields in the 1960s and early 1970s, indicated that these compounds were lethal to many amphibians (*64–66*). Today, organophosphates, carbamates, and synthetic pyrethroids are the insecticides predominately used in crop production. Thus far,

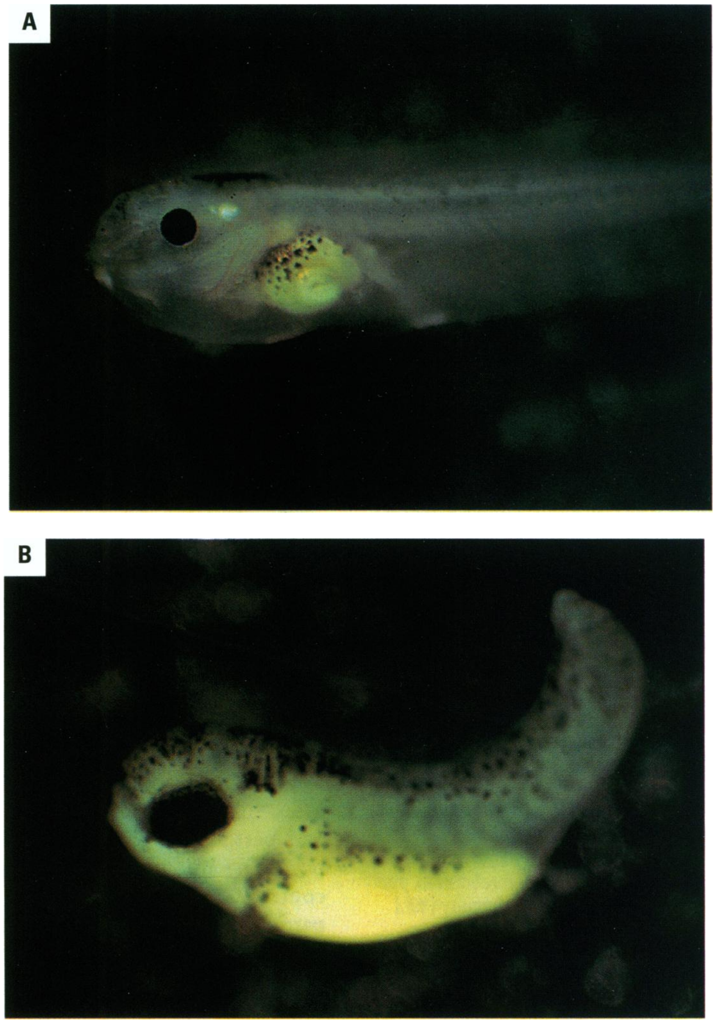

Figure 4. (A) Normally developed embryo at 96 hr (stage 46) and (B) severely malformed embryo of African clawed frog (*Xenopus laevis*) after 96 hr of exposure to agricultural drainwater from the Klamath Basin National Wildlife Refuges.

research on these compounds has demonstrated a range of effects, many of which are reversible or minor in whole organisms when exposed to concentrations of these compounds that are found in agroecosystems (*67–72*). However, researchers in Canada have recently used flow cytometry to assess frogs for effects from exposure to organophosphates. These researchers have observed increases in the coefficient of variation in the size of genomes in individual frogs as well as higher adult mortality and developmental malformations in the frog populations adjacent to the agricultural fields (Sharbel T, personal communication). Because information on the responses of amphibians to agrochemicals is limited, the need to assess amphibian responses to these compounds remains great (*3*).

Research on the effects of agricultural drainwater on biota has also been minimal (*73–75*) and was undertaken primarily because of concerns following the discovery of adverse effects of agricultural drainwater on the wildlife at the Kesterson National Wildlife Refuge in the San Joaquin Valley of California (*76*). After the discovery of selenium-related mortality and malformation among waterbirds at the refuge, reconnaissance studies of irrigation drainwater at 19 other western locations managed by the U. S. Department of Interior were conducted jointly by investigators from the U.S. Geological Survey, U.S. Fish and Wildlife Service, and the U.S. Bureau of Reclamation between 1986 and 1990.

Although the study of irrigation drainwater at Kesterson probably remains among the most publicized and extensive investigations to date, effects of the drainwater on amphibians were not assessed. In fact, none of the other 19 federal reconnaissance studies examined amphibian populations for adverse impacts from the agricultural drainwater. These studies included surveys at the Klamath Basin National Wildlife Refuges (*77,78*). The latter revealed that waters on the refuge have high temperatures, elevated pH, and low dissolved oxygen (*77,78*) (Fig. 5).

Subsequent evaluations of biological effects associated with water quality at the Klamath Refuges indicated that the irrigation drainwater was either lethal to, or caused significant malformation of, developing frog embryos (*79*). The agroecosystems surrounding the refuge use a variety of agrochemicals, including a number of herbicides and organophosphate and carbamate insecticides, for crop production. However, concentrations of these compounds in the water sampled were at or below detection limits (nanograms per liter). The study concluded that poor water quality (elevated pH and un-ionized ammonia) and/or pesticides may be contributing to the decline of indigenous frog populations (*79*). To our knowledge, this study was the first study of water quality and frogs in western agroecosystems.

Eutrophication. Elevated pH, low dissolved oxygen, high water temperatures, and elevated un-ionized ammonia levels characterize water in western agroecosystems and may singly or in combination have significant detrimental effect on the developing embryos of frogs. Elevated pH, ranging from 8.0 to 10.4, was recorded at Klamath Basin National Wildlife Refuges and has been recorded in waters at other wildlife refuges adjacent to agroecosystems in Colorado, Montana, and Wyoming (Osmundson B, Dickerson K, personal communications).

Ammonia is toxic to many aquatic organisms (*80*) and occurs in two forms in aqueous solution: the un-ionized form (NH_3) and the ionized form (NH_4^+). Ammonia equilibrium depends primarily on pH but also on temperature (*81*). As pH increases, the equilibrium moves toward the NH_3 form, and above pH 8.5, ammonia toxicity increases approximately 10-fold for each pH unit increase (*80*).

Water at the Klamath Basin National Wildlife Refuges contained NH_3 levels as high as 0.73 mg/L. Levels of this magnitude have killed fishes (*80–82*). Diamond et al. (*83*) reported an LC_{50} of 1.44 mg/L NH_3 for 96-hr acute tests with leopard frog (*Rana pipiens*) embryos at a pH of 7.14–8.21 and water temperature of 20°C. In the chronic test results, the leopard frog tadpoles were the most sensitive species with a no-observed-effect concentration of 0.27 mg/L (*83*).

In the case of salmonids, LC_{50} values for NH_3 range between 0.083 and 1.09 mg/L; for nonsalmonids, the range is 0.14–4.60 mg/L (*84*). The 19 species of invertebrates for which data were also reported have higher LC_{50} values (0.53 to 22.8 mg/L) than fishes (*84*). Further study of NH_3, pesticides, and other water-quality parameters in relation to frogs may elucidate the specific factors contributing to the

Figure 5. Agricultural fields border the waters of the Tule Lake National Wildlife Refuge on three sides and share water with the lake throughout the growing season. Many other wildlife refuges in the western United States receive irrigation drainwater as a primary source of water.

low frog populations on the Klamath Basin National Wildlife Refuges. These same factors may contribute to low frog populations in other western agroecosystems.

At Kesterson Refuge and in the Klamath Basin, the management of water within the agroecosystem upstream of the wildlife refuge has adversely affected water quality for fish and wildlife resources. From the beginning of agricultural production in this country, farmers have almost exclusively chosen monoculture crop production for short-term pecuniary gain (*85*). This singular focus has dramatically escalated during the 20th century and resulted in increased soil erosion, increased pesticide use, and water pollution (*56,57,60,86–88*).

For example, in a recent investigation, the U.S. Geological Survey studied water quality within the Pasco Basin of Washington State (*89*). Results indicated that the soils have become water logged and the groundwater levels have risen by 65 m during the past 35 years of crop irrigation. Many areas within the basin now require pumps to remove the groundwater from fields to protect crops from exposure to the water-logged soils. Simultaneously, nitrate concentrations in the basin water have increased as much as two orders of magnitude, and nitrogen fertilizers are the primary source of this change (*89*). Although these changes in water quantity and quality are now harming agricultural production in the basin, the impact on wildlife has not been studied.

Anthropogenic Determinants of Ecosystem Management and the Future of Frogs

Currently, no water quality criteria exist for amphibians in the United States. It is assumed that criteria for fishes and human health are adequate for protection of all aquatic species. This assumption, however, has not been tested. In view of the declines in the quantity and quality of water and amphibian populations in many parts of the United States, tolerance limits for amphibians need to be determined and compared with existing criteria for fishes and human health.

Postel (*56*) reminds us that we have been swift to claim water rights for our use, but slow to conserve quality or quantity of this vital resource for the needs of other species. Managers of metropolitan, agricultural, recreational, and industrial regions have largely aimed toward singular goals without consideration of the impacts of their activities on the larger landscape (*5,90–92*). Poor water quality is occurring on a global scale (*93,94*). Water is a resource that flows between and among ecosystems, permeating the larger landscape. Recently, Grumbine (*90*) suggested 10 specific themes for ecosystem management. Among them was a recommendation that managers depict boundaries at scales that are appropriate to the systems under management. Perhaps it is time for managers of specific regions or resources to consider the impact of their activities on the future of species other than humans. Grumbine also recommended interagency cooperation and organizational change to protect ecological integrity and biological diversity (*90*). Currently, the U.S Federal Wildlife Service and EPA are working together on water quality criteria for mammals and birds of the Great Lakes Basin. Perhaps amphibians will be next. In addition, the Declining Amphibian Populations Task Force (DAPTF) was established by the International Union for the Conservation of Nature in 1992. The DAPTF is an international network of researchers working to integrate specific information and studies of amphibian population declines.

Today, human populations control the resources on over half the land of the earth (*95*). All of the factors delineated above—habitat loss, introduction of exotic species, disease, overexploitation, global climate change, and water quality—are directly attributable to anthropogenic alteration of the landscape and climate resulting from the rapidly growing human population (*95*), a growth rate that is contributing to the increased rate of extinction of numerous species (*96*), including amphibians (*97*). If the human population continues to grow at its present rate, there will be even fewer resources available for all other species: less habitat, less water.

Protecting wetland habitat is critical, but alone it is insufficient for the survival and reproduction of wetland species. As stated previously, humans currently consume 65% of the available fresh water on the planet for agricultural use (*56*). As our population grows, we will claim even more of this vital resource for agricultural needs for food production. As the sharing and recycling of water increases, the quality of that water becomes vital to all consumers (*2,56,57,95*). Contamination by specific groups will affect all users. It seems both imperative and vital that protective water quality criteria be developed, acknowledged, and adhered to by all water users for the benefit and survival of all species that depend on this critical resource.

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